

nature

5 May 1977

'Nation shall speak peace unto nation'... at -30 dB

How should television develop in the next ten or twenty years? On the technical side, it goes without saying that most people would like to see every economically feasible development explored: cable television, satellite broadcasts, use of the receiver for access to news, data and computers, new types of television screen and so on. But on the question of programming there would be a variety of answers, as the recent Annan Committee has found in trying to formulate recommendations on Britain's fourth channel. Some want more of the same, some want educational programmes, some argue that more inevitably means worse. There is little doubt, however, that most (and this is not likely to be just a British view) would like more choice if there were some guarantee that this did not mean dilution of resources. One fascinating way in which this could have been achieved by enabling viewers to tune into other countries' programmes is likely to be suppressed before the general public is even aware of the possibilities.

At present, of course, television reception in the hundreds of MHz range is restricted by line of sight. The technology for transmission in the 11.7 to 12.5 GHz band from geostationary satellites is advancing rapidly, however, and there is talk in some countries of satellite broadcasting within fifteen to twenty years. At the same time, pressure is growing, but not in every country, for the allocation of frequencies in the same band to terrestrial services. An internationally agreed decision in 1971 gave priority to satellite broadcasting in choice of channels and this has meant a global gathering at Geneva earlier this year to allocate channels long before the precise needs can be foreseen. And since land-based services will develop well ahead of satellite broadcasting, the latter, now allotted its channels, will find itself in a straitjacket. The 20 MHz bands can certainly be used for radio as well as television, but a large screen digital receiver, which some describe as the television of the future, might well need greater bandwidth. It is not the technological straitjacket, however, which is most disturbing—it is the political constraint that programmes are not to cross frontiers.

As envisaged at present, each broadcasting nation would have its own satellite which would be at liberty to transmit on up to five channels, at 80 MHz spacings. The beams from satellite antennas would have a minimum width of 0.6° (which would more than cover Ireland) and could be elliptical (a beam $1.8^\circ \times 0.7^\circ$ would cover the UK). Every country in Europe, including Luxembourg, the Vatican, Andorra, Monaco, Liechtenstein and San Marino, has been solemnly awarded its five channels; in the case of the

smaller countries (in which there is arguably not quite enough talent to provide five simultaneous programmes and equally arguably adequate line-of-sight to surface transmitters), if they chose to launch a satellite the smallest beam possible would provide neighbouring countries with ample, if accidental exposure. On the other hand countries that wish to use one or more channels for deliberate broadcasting beyond their frontiers with broader-than-necessary beams have to obtain prior agreement from countries they wish to serve. The Scandinavian countries have been able to come to such an agreement, and the Vatican, Tunisia and Saudi Arabia (to serve Islamic countries) have also been allocated 'superbeams'. But other requests were turned down. Ireland wished to cover the UK for the sake of the large Irish population, but the UK claimed they had not had enough notice. And none of the major countries at the meeting seem to have made any public noises about broadcasting general television programmes over a wide area; indeed it is fairly widely known that the Eastern Europeans and France would refuse permission for such an invasion of their domestic life.

The objections are fairly obvious.

- Television could deliver some pretty slick propaganda; indeed it could, but the public on the whole switches propaganda off, and in any case an international channel might sensibly be compiled only of material which is also being shown domestically.
- There are technical problems of channel assignment and of having to direct an antenna at more than one satellite; hardly crippling problems in a technological age.
- The whole thing would only appeal to a tiny minority; well you wouldn't have to buy a multidirectional aerial if you didn't want to, and who is sure that foreign sport, music, entertainment and documentaries would not catch on? Twenty years ago Indian food and Spanish holidays were only for a tiny minority.
- We can already see foreign programmes; yes what 'the authorities' decide to accept on our behalf.

Too many conflicts in the past and too many prejudices in the present are based on the caricatures that most nations have of fellow-nations—caricatures largely fed by editorial control of the media. Here would have been a marvellous opportunity to ensure that at least the next generation could learn a lot more about the world as a whole—and maybe be stimulated to learn more foreign languages. But it has been thrown away. The next round of frequency discussions is probably ten or fifteen years in the future and will be too late. □



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Chris Sherwell reviews some of the problems in producing an EEC environment policy

THE achievements of international organisations are, in large measure, a political trade-off between what is theoretically desirable and what is practically possible. Unsurprisingly, the balance is usually in favour of the latter. In spite of, and perhaps because of their unique but complicating commitment to political and economic union, the nine member countries of the European Community (EEC) display the attribute more than most organisations. Nowhere is this more amply demonstrated than in environment policy.

The EEC's very involvement in the field is under challenge. When the European Commission last year produced its Second Action Programme on the Environment, covering the years 1977 to 1981, one of the most powerful of the environmental lobbies, the European Environmental Bureau (EEB), representing some 38 organisations throughout the Nine, weighed in with a powerful critique. There was a contradiction, it contended, between the main goal of the EEC, which was continuous and balanced economic expansion, and the basic principle of ecology, which was a dynamic equilibrium.

In fact the EEB displays all the signs of being pleased with the EEC involvement in environmental matters. The second action programme, after all, reads much like an environmentalist's manifesto, so breathtaking is its broad sweep. The EEB's reservations concern the precise character of that involvement. It criticises, perhaps with some justification given the programme's breadth, the curious lack of attention given to transport, population growth, regional policy and national programmes. This sort of gap, it is intimated, begs its own questions and reinforces the contradictions.

Questions are indeed necessary.

Environmental issues fall under an office in the Commission with split responsibilities—the Environment and Consumer Protection Service. Its staff numbers around 30. Environmental groups would like to see greater status attached to the department, perhaps making it a Directorate General. More importantly and perhaps contradictorily, they would like to see its work better integrated in the activities of the Commission and Community as a whole.

The argument is that the EEC's involvement in the environment will be ineffective if it is limited to drawing up a programme regardless of what the various departments of the Commission are doing, as now seems to happen. The aim ought to be to promote the concept of environmental quality in all the departments, so that the work done by an environment department is not emasculated by the activities of other departments. An environment protection department, in short, ought to participate in the policy making of other departments.

In spite of this very real structural problem facing the Commission, there are people who will praise its work; they recognise the constraints, both manpower and financial, that it faces in trying to coordinate the activities of nine countries across a broad range of environmental and other matters. But its achievements in such adversity are in turn the spring of other equally penetrating criticisms of the environment policy it administers. These emphasise the need for selectivity as a paramount requirement for success. The argument is that the lack of focus, the effort to do "too much too soon" and "run before being able to walk", is a fundamental cause for disillusion.

A glance at the second action programme is certainly enough to convince

even the most ambitious lobbyist that the chances of it being legislated in fifteen years, let alone five, are at best remote. This means that the sense of priority can become blurred, and emphasises the need for greater clarity about what the Community can do and what its role can be. After all, it is not just the Commission whose institutions may need modifying to encourage a well-rounded environment policy; it is also the Council of Ministers, before which the issues may come somewhat haphazardly and which finds the greatest difficulty in dealing with them systematically and without awkward and embarrassing haggling. The point is, not all environmental matters demand a specifically European approach.

Techniques of control

If the question of the degree to which the Community should become at all involved in environment policy seems important, it does not exhaust the list of fundamental questions on the subject which it currently faces. The very techniques of control are another issue, and form the basis for the longest running political controversy of all in this area.

Even boiled down, the essentials of the European debate are far from straightforward. The environment is recognised as having some capacity to receive pollutants. Where risks are apparent but complete prohibition is unnecessary, controls are needed. Two forms come under scrutiny, known in Europe as 'quality standards' and 'quality objectives'. Put most simply, quality objectives are pollution levels which the nine member states each undertake to do their best to attain, and quality standards are levels which the nine states each become obliged to achieve. The debate is about how precisely to use them.

Going by the political argument as it has developed thus far, the difference looks more than merely legalistic. For, having painstakingly arrived at a view of what the standards or objectives should be, the Commission is able to take steps to assist in their attainment by establishing what in its jargon are called 'norms'. This, it seems, is where the trouble starts, because it has meant the introduction of the notion of emission standards, to which Britain has strongly objected and other states and the Commission have given support.

The characteristic of emission standards causing Britain to dig its feet in is not that they are to be used, but that they are to be used uniformly. Britain has supported quality objectives on the grounds that they can take account of the varying nature of the receiving environment in a way that standards, because of their intrinsic

uniformity in the Community, cannot. To Britain, quality objectives imply emission standards but not uniform emission standards. Once it is agreed what quality a particular stretch of water, say, ought to have (which in turn depends on the use to which it is put), a quality objective must be set. To reach this, emission standards will need to be set for particular polluters on that stretch of water, but they will be peculiar to that stretch of water being used for that purpose.

Not so, say the hard-line Europeans. The imperfections of quality control mean that the only reliable path to reductions in pollution is to declare emission standards for particular classes or sections of industry and apply them uniformly throughout Europe; such standards would have to be achieved by a certain time. The British version of an emission standard, they say, is effectively a licence to pollute.

The arguments have in fact found their most coherent expression in the debate over aquatic pollution, itself just one aspect of pollution generally and a mere fragment of the environment question. Pointing to its shorter rivers, larger coastline and smaller and lower concentration of factories, Britain has argued that it can have pure water without adopting on a uniform basis the higher emission standards that would be demanded on mainland Europe. Other countries, with an eye to the Community's competition policy, have said this would give Britain an unfair competitive advantage in international trade and jeopardise a common policy both in competition and environment matters. Britain has contended that its advantage derives from possession of a natural resource and that a uniform policy would be wasteful, only harmonising costs rather than encouraging fair competition.

This dispute has occupied a considerable amount of important time. In part this is no doubt because of misunderstandings. For example, at a seminar organised in London recently on EEC environment policy, one Commission official cited the hypothetical example of two states in exactly similar circumstances trying to achieve the same air quality, in one instance by tackling only a few big companies and in the other by tackling many small companies; this, he said, would amount to an unacceptable distortion of competition. A surprised British official found that the example fitted with his own country's view of having the same environmental responsibilities in the same circumstances.

Much of the debate as it has involved aquatic pollution has necessarily involved the so-called 'black list' and 'grey list' of dangerous substances,

classified on the basis of toxicity, persistence and accumulation. Put most simply, the idea is to isolate black list substances from the environment to the point where there can be no doubt that they will harm human health, but some countries have said that black list substances should not be dumped or discharged at all. Others doubt the need for this, given the uncertainties about the substances themselves and about the absorptive capacities of the environment.

Although the fundamentals of the argument remain essentially unresolved, a breakthrough did come at a meeting of the Council of Ministers in December 1975 when a compromise solution was reached on black list substances. This embraced a dual approach allowing either emission standards on a uniform Community basis, or quality objectives which catered for different circumstances. Formally the compromise retained the notion of uniformity and allowed temporary exceptions for which Community but not uniform standards could be used: the exceptions would arise when a member state could prove that quality targets were being maintained throughout the particular area concerned, and would be re-examined every few years; the whole system would become operational within about three years, by which time priorities would be decided and actual objectives and standards set. Even then it might take five to ten years to achieve, and in light of this Britain views the application of the quality objectives as unlikely to lag much behind the implementation of emission standards.

For the grey list it was agreed that individual member states would prepare programmes which included quality objectives, from which they would derive emission standards of their own. The Commission would ensure that comparisons between countries were made to ensure some uniformity of approach, but the underlying approach became one involving quality objectives, and there is no mention of emission standards on a uniform Community basis. The recent seminar in London may anticipate an even broader acceptance of the British view.

Fears persist that the black list will in effect become a grey list. However, the detail of the breakthrough, though important, was less important than its immediate consequences. Progress could be made in April 1976 towards a Rhine convention covering pollution by salts, chemical pollution, thermal pollution and pollution by radioactivity. Agreement on limits of chemical pollution was soon reached, and a month later ministers met again to resolve

differences over salt pollution. Progress was also possible on the matter of Mediterranean pollution. In Barcelona in February 1976 16 countries finalised details of a framework convention and two protocols covering the discharge of petroleum waste and the tipping by ships and aircraft of black list and grey list pollutants into the Mediterranean.

Achievements not matched

These 'international' achievements involving Community members have not since been matched within the Community itself. At the most recent Council of Ministers meeting last December, for example, four draft directives from the Commission awaited approval: on the biological surveillance of the population for lead in the blood; on the quality of water intended for human consumption; on the waste from the titanium dioxide industry; and the reduction of water pollution by paper pulp plants. The Council adopted only the first of these.

At the end of last year, in fact, there was still around a dozen sets of proposals for directives sitting on the Council's desk awaiting action. They included proposals relating to the lead content of petrol (submitted 7 December 1973), the dumping of wastes at sea (submitted 12 January 1976), health protection standards for sulphur dioxide and suspended particulate matter in urban atmospheres (submitted 25 February 1976) and toxic and dangerous wastes (submitted 28 July 1976). Proposals for decisions awaiting adoption included one on the subject of organic micro-pollutants in water and another on exchanging information on the quality of surface fresh water in the Community.

This last item is expected to appear on the agenda, along with the other undecided items from the December meeting, when the Council of Ministers meets in June for the one and only time during Britain's present chairmanship. Outstanding items will probably also be included, and perhaps some new ones like the conservation of birds, though the chances of them being considered remain as always subject to the will of the member states on the higher

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priority items.

In its programme for 1977, published in February, the Commission says it will concentrate on four basic 'priority themes': measures to combat water pollution; evaluation of measures to prevent the deterioration of the environment; an anti-waste campaign based on a policy to encourage materials recycling; and implementation of the international conventions on the Rhine and the Mediterranean. It says it will therefore send to the Council proposals for directives on, amongst other things, protection of underground water, the quality of water for agricultural use, notification of industrial

activity involving dangerous substances (a lesson of Seveso), the discharge of various particularly harmful pollutants, and on anti-noise measures.

The Commission thus remains optimistic. Its work goes ahead under the auspices of the second action programme, which the Council meeting of last December unanimously approved, adding the undertaking that it would act on concrete proposals from the Commission within nine months of them being forwarded. That may prove to be a tall order, given the fundamental character of some of the issues still outstanding concerning the Community's environment policy. But the

Community and its policies are nothing if not resilient. And the large number of directives which have already gone through suggest that whole effort in the environmental field may have acquired a momentum.

That should allow some attack in the primary field of preventing and eliminating pollution and nuisances, where most attention has been concentrated. But managing the use of natural resources properly, controlling the ecological balance and economic growth, and generally protecting the biosphere—also aims of the EEC's second action programme—will plainly take a little longer. □

Spreading the word

David Spurgeon, recently in Asia, examines the growth of science writing there

THE business of writing about science for the mass media must have its ups and downs like any other business. Although it is difficult to judge without the evidence of a full-scale survey, it seems the trend in North America in recent years has been predominantly down. Editors no longer show the enthusiasm for science subjects that they did during the early post-Sputnik days and discussions have appeared in science writers' newsletters about the parlous state of their craft.

Some link the decline in interest to the scepticism—and in some measure, disillusionment—about the promise of science that followed the excesses of publicity associated with the US manned space programme, the development of nuclear power and 'wonder drugs' and other achievements. It seems a reasonable thesis; the earlier 'gee-whiz' phase of science writing was followed not only by a much more critical and judgmental phase, but also by the birth of various anti-science movements and by 'consumerism' and 'environmentalism'.

An interesting contrast is found in the Third World, particularly in Asia, where (again to some extent subjectively) interest in science journalism seems definitely on the upswing. Mack Laing, a Canadian science writer and professor of journalism, writing in Depthnews Science Service, a weekly newsfeature service of the Press Foundation of Asia (PFA), put it this way in a recent article from Manila:

are going full blast on the idea. Leaders in these countries argue that popularising science encourages people to understand and cooperate with government actions on large-scale problems such as conservation, pollution, irrigation and flooding by man-made reservoirs, sanitation and infectious diseases. They argue that interesting the youth of a country in science increases the country's science manpower and speeds national development. One way of bringing the word about science and technology to the people is through the media of mass communications.

The Depthnews Science Service and Mr Laing's activity in it are themselves interesting results of this newly-awakened interest. PFA, a non-profit agency founded by Asian journalists some ten years ago to improve the standards of the region's media, asked Canada's International Development Research Centre (IDRC) for funds to set up a science feature service within Depthnews. Its aim would be to set the pace for the Asian press in science coverage, which in its opinion (and that of other Asians) had been deficient until then. PFA also asked for an experienced science journalist from the West to set it up and train Asians in

the techniques of science journalism.

While it is too early to judge the commercial viability of the science feature service, the initial response of the Asian media has been surprisingly good. Mr Laing has found himself in the ironic position of having to admit that the interest shown by the media in Asia for his service's articles is much greater than he could imagine Canadian editors showing for a comparable service at home.

Among his chief sources of news in the Philippines are weekly news conferences set up specifically for science writers by the University of the Philippines and the National Science Development Board. The conferences were arranged to acquaint science writers with researchers whose work is sponsored jointly by these two organisations. It seems to be succeeding. At a recent conference, about ten media representatives gathered in a small room to hear a pharmacologist talk about work she and her colleagues had been doing in identifying plants with insecticidal qualities that could serve as replacements or alternatives for chemical pesticides. (The other subject, oddly enough, was music, but apparently this was atypical, almost all subjects being scientific).

To encourage attendance, the university has adopted the practices of paying the journalists to attend the con-

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There is a burst of activity in Asia these days toward achieving a greater public understanding of science. Some—perhaps even most—Asian nations are just waking up to the need for more public appreciation of how science and technology can help national development. Other countries

Ernest Corea

Sri Lanka seminar on popularising science, fiction writer Arthur Clarke speaking

ferences, which are held on Saturday mornings, and of giving them coffee and lunch. The sum is small (the equivalent of about \$9) and although the practice would obviously be unacceptable to the Western press, it may help (and perhaps be necessary) in the Philippines. Dr Joventino Soriano, the coordinator, first introduced the news conferences by holding a four-day science writers' seminar at a mountain-top retreat some miles from Manila. A travelling seminar was also planned in April, when media writers and researchers were to visit villages in three provinces north of Manila to see how science and technology can aid rural areas.

Similar seminars have been held by other organisations. In 1974, the IDRC held one near Manila for science writers. The response was strong from the media in eight regional countries, some of which already had science writers (and one, Korea, highly developed science coverage). In other countries (for example Indonesia) the interest was there but the media structure was not. In 1975, another seminar was held in India, with a similarly strong response from that country and others bordering it. A third seminar planned for June in Nairobi, will be for the East African media.

In March, a month-long Asian Training Course for Leaders in the Promotion of Public Understanding in Science, sponsored by UNESCO and the Science Foundation of the Philippines, was concluded in Manila. And in February, as a result of the previously mentioned India seminar, a meeting was held in Colombo, Sri Lanka, on 'Science writing for the people'.

In Sri Lanka, where science writing is largely ignored in English-language newspapers, possibly partly because newsprint shortages make for thin newspapers, the Ministry of Education is reported to be planning on providing scholarships for science writers to obtain university degrees.

At the recent seminar, Professor P. P. G. L. Siriwardene, the Vice-Chancellor of the University of Sri Lanka, said that science must be written in the vernacular to get to the people. But he added that popularisation of science has been "hamstrung by a lack of funds". In the developing countries, he said, science popularisation should be "an important national venture". The question of language is an important one, as delegates to the IDRC's first seminar noted. In many Asian and African languages there are no words for some scientific

concepts. However, in Sri Lanka, as in India, some science does appear in the vernacular-language press.

The Philippines is one of the most enthusiastic promoters of science popularisation among Asian countries. In addition to the emphasis on science news (which is perhaps attractive to editors because of government control of the country's press and the dearth of genuine news stories) there are hundreds of science clubs, which are joined in a federation and hold Youth Science Camps. Science campers visit rural areas to explain science and scientific concepts to farmers and remote villagers, and to replace superstition and myth by rational explanations of natural events. A science quiz, an annual high school contest, has attracted 8,530 regional participants and 292 national participants since 1969.

In a speech prepared for the course on public understanding held in Manila last March, President Marcos said:

We are belatedly finding out that the harnessing of science and technology is not a mere matter of learning formulae, but a process of cultural transformation itself. It is in this light that I perceive the importance of promoting wide public understanding of science, technology and the environment. □

RED SEA

Sifting the sediment

The staff of La Recherche reports on the prospect of mining sediment in the Red Sea

A CONTRACT was signed on 25 March, in Paris between the Red Sea Commission, represented by its secretary general, Dr Zaki Mustafa, and the Argas Company, represented by its president, Jamil Jawa. The contract, involving FF30 million (£1=FF8.54) is one of a series of agreements on the exploitation of the metalliferous sediment in the Red Sea, which contains 5.8% zinc, 0.90% copper and 110 g of silver for every ton of dry material as well as iron and manganese.

The first agreement between Saudi Arabia and the Sudan, signed in May 1975, was to cooperate in exploiting the mineral resources at the bottom of the Red Sea in the so called 'Common Zone', which lies between the two countries. The resulting Red Sea Commission, which has its headquarters at Jedda, was founded to promote and encourage mining research and any means of exploiting the minerals in the zone. The Commission then called in the Argas Company (The Arabian Geophysics and Surveying Company, a joint

venture between the Saudi company Petromine and the French Compagnie Générale de Géophysique).

Two preliminary contracts in March and July 1976 (for FF15 million and FF8 million respectively) covered geochemical, seismic and aeromagnetic studies. The work to be carried out under the third contract, the one recently signed, will analyse the environment including swell, currents, thermosalinity and biology to ascertain the effect of exploitation on the environment and to determine the conditions necessary for lowering a vertical tube for pumping mud to 2,200 m.

The metal extracted from the sediment would be treated on the spot, and mineral salts and tons of residue extracted from the metal ore would be thrown back into the sea; but what would become of this residue, and what effect it would have on the flora and fauna before settling back on the sea bed remain unanswered questions. A German company, Preussag, has meanwhile been developing the extraction process. A pilot preconcentration plant could be working by 1982, and it will not be known until then whether the project is feasible. Preussag is also under contract from the Red Sea Com-

mission, which is providing \$25 million for six years. The Commission has engaged the services of a French public body in charge of geological and mining research, the BRGM, to coordinate activities and act as technical adviser.

If the project succeeds, it will be the first time that marine sediment has been put to use: other companies engaged in working the ocean bottom are more interested in nodules. The sediment contains important components, however, especially copper, and similar deposits could be found in other parts of the world. Geological studies provide an explanation for the formation of mineral rich sediments: in the Red Sea, where rock outputs cut across the rift, there is a zone of intense volcanic and thermal activity, and the rocks give off large quantities of metallic salt into the water. In the presence of the hot brine frequently found in these regions the metallic salts are eventually precipitated. Thus a sedimentary bed is formed containing enough metal for it to be thought worth while mining out. The first mining will be in the Atlantis II fault, where it is estimated that there would be 850 million tons of sediment, that is 7×10^6 tons of copper. But at least 18 faults are known in the hot brine regions of the Red Sea; some probably do not contain sediment, but others could be extremely profitable. □

USA

Going public on rights

Colin Norman reports from Washington on an appeal in support of named scientists who are victims of political repression

In a significant departure from its usual style of quiet diplomacy, the National Academy of Sciences last week called a press conference to urge public support for eight scientists in three countries who are "victims of grave official harassment for political reasons". The action, taken by a newly-established Committee on Human Rights, signals that the Academy is prepared to adopt a more aggressive role in defending scientists against political repression than it has in the past.

The eight scientists involved in the appeal are five Argentinian physicists who disappeared in 1976, a Uruguayan mathematician arrested in October 1975, who has been charged with subversive activities and allegedly tortured, and two Soviet scientists, one of whom is serving a prison term and the other is under arrest awaiting trial. According to Robert W. Kates, the chairman of the Academy's Committee on Human Rights, these eight scientists are the first of "a large group" which the Academy hopes eventually to take public action on. "They are", said Kates, "at best symbolic of the many scientists who are subject to repression".

The Committee on Human rights was established a year ago, following complaints from some Academy members and from the Federation of American Scientists (FAS) that the Academy had been too timid in its attempts to defend scientists against acts of political repression. Though it made one highly publicised statement in support of Andrei Sakharov in 1973, the Academy has worked mostly behind the scenes, making private representations to officials of foreign governments on behalf of harassed scientists. Early last year, there was a move, sparked by complaints by the FAS Director, Jeremy J. Stone, to urge the Academy to back up its private negotiations with occasional public statements.

The Academy responded by establishing the Committee on Human Rights and it also adopted a set of guidelines which suggested that the Academy would speak out publicly when the occasion so demands. It seems from last week's press conference that such public appeals may become more common. Kates said that the committee will concentrate on in-

dividual cases rather than making broad statements of principle, and he indicated that several more cases will be added to the list as soon as further evidence is collected. "This is just the beginning", he said.

Asked whether the committee would be prepared to urge the Academy to take further steps if the appeal has no effect, Kates said that the committee would proceed one step at a time. The ultimate sanction available to the Academy would be to back out of an exchange agreement with a country which persists in acts of political repression against scientists. In any case, there seems to be a strong body of opinion within the Academy supporting the committee's moves so far. Kates said last week that a letter sent to Academy members asking for their support in writing to foreign officials to protest acts of political repression produced 258 positive responses.

The specific action taken last week was to ask scientists to write to officials in Argentina, Uruguay and the Soviet Union in support of the eight scientists.

In Argentina, the five physicists are Federico Alvarez Rojas, Gabriela Carabelli, Juan Carlos Gallardo, Antonio Misetich, and Eduardo Pasquini. All disappeared during 1976, presumably arrested, but the government claims to have no knowledge of them. The Academy is asking that they be permitted to see their families and that scientists be allowed to visit them to determine their state of health. In addition, the Academy is calling for the physicists either to be charged or released and that observers be permitted at any trial. In fact, however, members of the Academy's committee said that there is no guarantee that the five are even alive.

The Uruguayan mathematician, José Luís Massera, was arrested in October 1975, held incommunicado for a year and is now being tried in camera on charges of "subversive association". According to Lipman Bers, a member of the Academy's human rights committee, Massera, who is 62, is "the most distinguished mathematician in Uruguay" and he has a worldwide reputation. An avowed Communist, Massera has been "subject to severe torture", Bers said. In this case, the Academy is again asking for permission for Massera's family members and representatives of the world community of scientists to visit the mathematician, for observers to be permitted at his trial, and for him to be given access to books and other scientific material.

The two Soviet scientists are Sergei Kovalev, a biologist who is now serving seven years' hard labour and three years' exile for alleged activities against the Soviet state, and Yuriy Orlov, a physicist who was arrested in February 1977 and is awaiting trial on as yet unspecified charges. In Kovalev's case, the Academy is concerned about his ill health, and is appealing for commutation of his sentence to the time already served. The Academy is also requesting scientists to appeal directly to the Soviet authorities on Kovalev's behalf and to provide Kovalev himself "with every feasible manner of moral and professional support". As for Orlov, he has been a leading participant in the Soviet human rights movement associated with Sakharov, and was arrested as part of the recent crackdown on the dissident movement. The Academy is inquiring about his legal status and place of detention through a letter sent last week to the Soviet Ambassador in Washington, Anatoliy Dobrynin, and the human rights committee will "take appropriate action".

The Academy's new, more aggressive policy puts it in marked contrast to the Royal Society. In a speech to Royal Society Fellows last November, Lord Todd, the Society's President, expressed concern about the widespread political repression of scientists in many countries and about more subtle infringements of scientific freedom, but he said that the Royal Society should steer clear of making public pronouncements on the matter. Instead, Todd said that Royal Society officials would continue to make private representations on behalf of scientists whose human rights are being infringed.

Further details of the individual cases raised by the Academy last week, and addresses of government officials to whom correspondence should be addressed, can be obtained from the Committee on Human Rights, National Academy of Sciences, 2101 Constitution Avenue NW, Washington DC 20428. □



Sergei Kovalev

USSR

● The raising of quality standards is a major watchword of the current Five-Year Plan and a slogan for the forthcoming celebrations of the Diamond Jubilee of the Soviet Union. In the traditional panegyric of Soviet achievements given on Lenin's birthday (22 April), Mikhail Zimyanin, Secretary of the Central Committee of the Communist Party of the Soviet Union, noted among other achievements an improvement in the quality and efficiency of research and the coordinated study and solution of problems of scientific and technological progress. A major re-equipping of industry is under way, he said, and in spite of "difficult weather conditions" the last year has been marked by "new outstanding achievements of the Leninist agricultural policy of the Party".

Nevertheless, in at least some of the Union Republics, there is less emphasis on the implementation of the plans. In Lithuania, according to the First Secretary of the Central Committee of the Lithuanian Communist Party, there is a tendency for certain ministries and departments to draw up "easy" plans and adjust the targets downwards. The First Secretary of the Central Committee of the Byelorussian Communist Party, speaking at the recent Plenum, told a somewhat similar story. Some bodies including research institutes and the state statistical organs, he alleged, keep up a front of activity, busying themselves with data-gathering and the preparation of summaries rather than with the "scientifically substantiated recommendations" which the economy requires; even when recommendations were made, the proper experimental basis might be lacking.

He cited in particular a new system of product quality control for dairy farms which was recently worked out by the Byelorussian Scientific Research Institute of Agricultural Economics and Organisation and which has been widely publicised. However, the whole experimental basis of the scheme apparently rests on "a brief experiment on only one farm", reflecting a lack of practical trials reminiscent of the extremely scanty foundation for Lysenko's "vernalisation" scheme. Although not criticising in so many words the system of targets which is the basis of Soviet planning, the Byelorussian first secretary deplored the tendency to set targets without proper evaluation of the "qualitative parameters of economic development".

● The Dubna Joint Institute for

Nuclear Research has completed a cycle of experiments on the synthesis of element 107 by bombarding ^{209}Bi with nuclei of ^{54}Cr . The first experiments, in 1975, gave strong evidence of the formation of the element with a half-life of 2 milliseconds. The Dubna team, under Academician Georgii Flerov, has already syn-



thesised elements 103–106. In announcing the successful conclusion of the current series of tests, Academician Flerov gave a strong hint that they now would be aiming at elements of even higher atomic numbers—a new unit is being built, he said, which will increase the efficiency of nuclear interactions by a factor of several hundred.

● One of the major features of recent Soviet work in the Antarctic has been the drilling of deep cores both through the ice-shelf and on the continent proper. A special electrothermal core drill is used, with a maximum penetration of 6 metres an hour with, when necessary, a special system for keeping the core sterile. All apparatus is easily transportable by sledge or helicopter, hence cores may be taken if required at a considerable distance from the base.

The ice-shelf project was based on the Novolazarevskaya station (Queen Maud Land). The purpose of the experiment was not simply to penetrate the ice but also to sample the sea-water and sea-bed below. The first such shafts gave the depth of water beneath the ice as 40 m, its salinity approximately that of the open ocean and its temperature -1.5°C to -1.9°C . The sea-bed specimens indicated that the composition of diatoms deposited had not

changed during the past 10,000 years.

Inland drilling operations were based on the Vostok station at the 'Pole of Cold', where the icecap is some 3,500 m thick. A 980 m core from this area has been analysed by the ^{18}O method. According to Professor Evgenii Korotkevich, who is one of the supervisors of this project, the core indicates a palaeo-climatic pattern with a mean annual temperature at 15,000 B.P. some 5° below the present figure, indicating a significant drop in the world temperature. This, he says, was followed by "rapid reconstruction" of the climate, thought to be associated with cosmic phenomena, with the present climatic conditions becoming established *circa* 11,000 B.P., since when the climate has remained virtually unchanged.

The Vostok station is also the base for taking sterile cores for experiments on the 'reanimation' of microorganisms. The cores from the twentieth expedition (Summer 1975–76) penetrated to 207 m, the most ancient organisms reanimated being from the 197 m level ($\sim 8,500$ B.P.). From the specimens of the twenty-second expedition (Summer 1976–77), it is hoped to establish the limit of survival of microorganisms in the anabiotic state, a topic, it is said, in which the Laboratory of Space Microbiology of the Microbiological Institute of the Soviet Academy of Sciences is showing considerable interest.

● The case of Anatolii Shcharanskii, at present under arrest pending investigation, is a peculiarly ironic example of the 'secrecy' issue which faces scientists wishing to emigrate to Israel. Shcharanskii, who is prominent both in Jewish refusenik circles and in the larger human rights movement (he is a member of the banned Helsinki monitoring group), studied at the Moscow Institute of Physics, specialising in computer science. He has not, however, ever worked at his profession.

Because the research post he was offered at the Physics Institute of the Academy of Sciences could conceivably have been considered as 'classified', thereby threatening his chances of emigration, he denied himself the possibility of creative work in his own field and was employed as an engineer. The latest Tass allegation that Shcharanskii has been organising the collection of classified information to send "abroad" thus adds a bitter twist to his story.

Vera Rich

IN BRIEF

Brain drain persists

Although the brain drain has been partially plugged by restrictive immigration regulations in the United States, it is still proceeding apace, according to a study released by the National Science Foundation (NSF). Between 1966 and 1975, some 10,000 scientists and engineers emigrated to the United States, the study indicates, though the average annual influx dropped from 11,500 to 6,500 during that period. The two chief influences were an immigration regulation adopted in 1965 which eliminated the preference previously given to European applicants for immigration, and a regulation adopted in 1971 which removed scientists and engineers from the priority list because of high unemployment among scientific workers in the United States.

The result has been an overall drop in the number of scientists and engineers entering the United States, coupled with a marked shift in the relative numbers coming from Europe and Asia.

In 1965, the ratio of scientific emigrants from Europe and Asia was 40% and 28% respectively; by 1975, it had swung to 19% and 56%. Throughout the decade, the ratio of engineers to scientists remained fairly constant at about 2 to 1.

Waste report

The UK National Radiological Protection Board (NRPB) has followed up its recent publication dealing with disposal of high-level radioactive waste on the sea-bed with the release this week of a report studying the ways low-level waste is dispersed in the ocean (NRPB-R58, HMSO, 50p). Disposal of such waste, which consists of contaminated equipment, clothing and rubbish packaged with concrete or bitumen in metal drums that eventually corrode, has gone ahead under international arrangements for some years. The report describes a two-part (short term and long term) mathematical model for estimating maximum con-

centrations of radioactivity in water for continuous disposal, and can be used to assess the ocean's limiting capacity to accept radioactive waste.

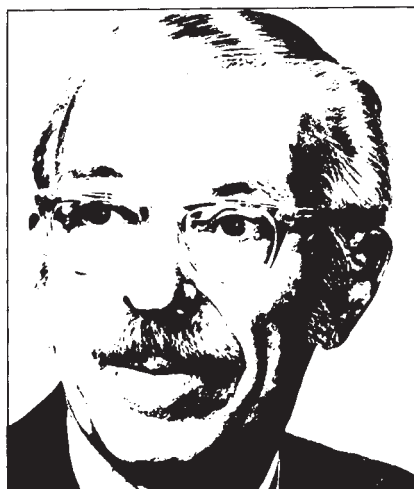
Geos switch-on

Switch-on of experiments on Geos started on 29 April. Five out of the seven experiments have so far been tried and are working; the boom deployment programme is progressing satisfactorily. Eight hours of effective data transmission per day will be possible but only about four hours will be beyond 5 earth radii where the experiments are designed to operate. At a longitude of 35° E next week Geos could interact with Northern Scandinavian ground stations, but very little useful data are expected because of the movement of the satellite. Only a few per cent of the original data-taking capabilities of the mission can be realised, putting the IMS in jeopardy. A prompt launch of the spare Geos remains desirable.

EARLY in April the Federation of American Societies for Experimental Biology met once again in Chicago. It was, as always, the windy city, with snow flurries blowing off Lake Michigan. I stayed in the Conrad Hilton Hotel, as I had in 1941, when it was the Hotel Stevens. Some years later, I think in 1956, I had squeezed my way into a doorway of a packed room to hear a paper by Volkin and Astrachan on 'DNA-like RNA'. It was one of the first premonitions of messenger RNA. Prior to this, ribosomal RNA seemed the only possible template for protein synthesis, and its uniformity of composition in different organisms was not compatible with the diversity of their proteins. This year a capacity crowd of thousands sat comfortably in a big new theatre to hear a virtuoso performance by Fred Sanger as he described the nucleotide sequence of Φ X174 containing instructions for 1,849 codons in genes for eight proteins. One of the audience told me afterwards that it was always a humbling experience to listen to a lecture by Fred.

My thoughts turned to Federation Meetings, years ago, when a livelier iris changed upon the burnished dove and sprightly young women scientists caught my bachelor eye, or I tried to catch theirs. Alas, in 1977, I found myself looking automatically at all people with grey hair because I expected to recognise them as my contemporaries; souls that have toiled, and wrought, and thought with me. Such are the dividends of advancing

years. But there are really few experiences so rewarding as that of greeting an old colleague and friend whom one has not seen for a long time.

Federation meetings

THOMAS H. JUKES

I was the final speaker in an evening session in a meeting room at the Conrad Hilton Hotel. A modern note of social disintegration was sounded by an incident that took place within five minutes after I finished; someone stole the slide projector. I never found out if the purloiner was a disgruntled scientist who sought belated revenge because his slides at some meeting had been shown upside down.

The 'poster sessions' in the

McCormick Place Exhibit Hall were obviously very successful. This format has increasingly replaced the old system of 12-minute papers read in an often crowded and usually darkened room. On Tuesday afternoon, for example, there were 13 'regular' sessions in biochemistry and 9 poster sessions. The authors are on hand at their assigned posts, complete with posters, graphs and abstracts, to discuss their work with all passers-by who are interested. This gives much more opportunity for an exchange of views than is afforded by two or three minutes allowed for questions after a regular paper. The Exhibit Hall was, as ever, the scene of a dazzling profusion of intricate biochemical equipment and apparatus. One would not have suspected any slow-down in research.

There was the usual profusion of diverse social gatherings and cocktail hours, ranging from the Annual Meeting and Wine Mixer Association for Women in Science to the Korean Biologists Tea and the Physiology of Exercise Mixer.

I am afraid I have very little sympathy with those who oppose the granting of travel funds for scientists to attend such meetings. At such gatherings, names become changed into people, ideas are exchanged and stimulated, and long friendships are started. The public has garnered sufficient dividends from the progress of the biological sciences in the past 50 years to support the continuation of the Federation Meetings.

news and views

New accelerators for high energy physics

from W. J. Willis

The super proton synchrotron (SPS) at CERN in Geneva is due to be inaugurated officially on 7 May. In this and the following two articles practicing scientists look at future prospects.

A NEW stir of activity among physicists studying elementary particles has become unmistakable. In part due to the excitement generated by the suggestive discoveries of the past 3 years, it has also to do with the sense that visions of important future facilities may become real. In January the SPS, a 400 GeV proton accelerator was commissioned for physics at the CERN site near Geneva, bringing to completion the generation of facilities planned in the 1960s. While these are exploited, discussion of possible future steps grows more serious.

Extension of existing facilities

Some important and interesting developments of the existing facilities are already underway, speeded by competition, the excitement of the new discoveries, their relatively moderate cost, and the fact that complicated institutional arrangements were not required. Two similar storage rings to study the collisions of beams of electrons and positrons at energies up to more than 20 GeV are under construction at the DESY Laboratory, near Hamburg, and at SLAC, in Stanford. The German machine, PETRA, is expected to be completed in 1979 with the Californians' PEP some months behind. The recent experiments on e^+e^- annihilation in the few GeV region led to the rapid discovery of many new particle states and showed that this is a clean and powerful way to study the creation of states containing many strongly interacting particles. The notion that a good many of the discoveries will be made quickly, and the similar schedules for the two machines, have combined to give their construction somewhat the character of a race.

Another ongoing project is the Energy Saver-Doubler at Fermilab, a characteristically bold endeavour which represents the first commitment to build

and operate a large number of superconducting magnets. These will be placed in the existing tunnel and the protons transferred to the new ring at an energy of several hundred GeV. The risk is moderated by the consideration that the project will be justified, as an Energy Saver, even if the magnetic field initially attains only the 2.2 Tesla now provided by the conventional magnets. Of course it is expected that the superconducting magnets will reach fields greater than 4 Tesla, allowing the new ring to accelerate protons to 1 TeV (1,000 GeV). The necessary strengthening of the experimental beams and shielding would then allow a new experimental programme at higher energies and perhaps intensities. The schedule and cost are not firm, but the experimental programme at 1 TeV is certainly several years distant.

These developments open the way to many new kinds of experiment, but it should be pointed out that they do not provide higher centre of mass energies than those already available from the collisions of the two 31-GeV proton beams of the CERN ISR. This falls short of the region of 100 GeV of available energy, where the weak and electromagnetic interactions may reach the same strength. The recent discovery of neutral weak currents supplies a missing link in the argument that these two interactions are different aspects of a single fundamental phenomenon. It is widely believed that when the necessary energy is reached, their unity will become apparent, probably by the creation of a new family of very massive particles which are the quanta of the weak electromagnetic field. In testimony before a Congressional committee several years ago, T. D. Lee drew an analogy between the consequences of this hoped-for unification and the unification of electrical and magnetic forces achieved by Faraday and Maxwell. In this context the PEP-PETRA race can be viewed as an exciting skirmish on the way to the goal sought for so many years.

Colliding beams and electron cooling

Attempts to reach this goal are foreseen on two levels. Both Fermilab and

CERN hope to reach centre of mass energies well above 100 GeV by colliding beam experiments based in large part on the use of existing equipment. At Fermilab, one possibility considered is to store protons in the Energy Doubler at energies up to 1 TeV, and collide them with protons in the existing Main Ring at about 200 GeV, giving centre of mass energies up to 900 GeV. Also, both laboratories are planning to continue the investigation of the technique of "electron cooling" invented and successfully demonstrated by G. Budker in the Soviet Union, to allow the storage of large currents of antiprotons. In this technique, an electron beam is passed through a beam of antiprotons created by the accelerator and captured in a small storage ring. The antiprotons are created with a spread in energy and angle which causes them to fill the aperture of the storage ring. The electron and antiproton beams, which have the same v velocity, are coupled by the Coulomb force. The antiproton beam exchanges its disordered motions with the constantly renewed electron beam, and is damped into a narrow beam occupying only a small part of the storage ring aperture. More antiprotons are injected and the process repeated over many hours, until a large current of antiprotons is built up. These are then injected into the large ring, together with a counter-rotating proton beam, and accelerated to the maximum energy. Apart from a possible advantage of antiproton-proton collisions in creating new states, the noteworthy feature of this scheme is that it requires but a single large ring, and thus allows the conversion of an existing accelerator into a colliding beams facility. This has long been the aim of Budker's group at Novosibirsk, viewed with a certain scepticism by the orthodox accelerator scientists until the recent success. A very different cooling method has also been demonstrated recently at CERN.

In the forthcoming tests, CERN has the advantage of using an existing small storage ring, but the ring Fermilab will construct for tests with protons can also be used for antiproton

accumulation in the final scheme. If all goes well, these efforts could lead to colliding beam experiments in the early 1980s. If this technique is applied to the Energy Doubler, we might see collisions with 2 TeV in the centre of mass in 5 years or so.

Plans for future facilities

These heady visions must be balanced by some cautions. Major extrapolations are involved in these designs, and the event rates which will actually be achieved are most uncertain. The experiments in the region where the weak and electromagnetic interactions become equal are not easy, and involve quite small cross sections. It may be that these projects reach success without being able to perform these difficult experiments in a conclusive manner. In any case, these are attempts to carry off a coup, and will not yield facilities where many experiments can be carried out simultaneously. The more conventional plans to reach the energy goal may prove to be the more important for physics. The discussion of major new facilities proceeds primarily within the traditional framework of the USA, the European collaboration, and the Soviet Union, but efforts to establish a world-wide collaboration have begun to have effects, mainly indirect. The "world accelerator" discussion has been carried out off and on since 1960, but achieved its most interesting stage last May in a meeting at Serpukov. The participants agreed on two points. They undertook

to avoid a needless duplication of facilities. Whether this is an admission of guilt in the PEP-PETRA case is moot, but it has real implications for the next step. It was further agreed that an attempt would be made to fix upon a world facility in about 10 years' time, leaving enough time for one more round of "regional" facilities. The International Union of Pure and Applied Physics was assigned the task of studying some organisation for further work, with US, European, Soviet, and Japanese participation. (A rapid cycling proton accelerator of 10 GeV is now working in Japan.)

The reality of the world accelerator may be uncertain, but the fixing of a kind of deadline for any large regional effort has immediate consequences. As it happens, the US has a mature proposal ready for a decision. This is ISABELLE, a superconducting proton storage accelerator of 200–300 GeV, to be built at Brookhaven, and given a good chance of gaining approval soon. Partial approval has apparently been granted at Serpukov for an accelerator of 2 TeV, called UNK, to be converted to storage rings later. In Europe, it has not been possible to carry out detailed planning for a new facility while constructing the SPS, an expensive instrument whose approval and financing has already created tensions among the CERN collaboration. This is an awkward time to seek bilateral arrangements with the US, since Brookhaven is confident of obtaining approval without further complications.

This situation, and the 10 year deadline, has led to a sense of urgency among European particle physicists. Luck seems to be with them, since the great success of the electron-positron colliding beams has revealed a big gap in the world plans, in that there has been no plan for an e^+e^- machine to reach the goal of 100 GeV or greater, though it is probably possible to build such a machine at a reasonable cost. If the neutral weak currents arise from a heavy particle with a mass of about 80 GeV, this machine would be a superb tool for investigating many new phenomena, and it is in any case a powerful complement to the proton collisions. This idea is now under study by the European Committee on Future Accelerators, and by CERN, along with the LSR, a 400 GeV proton storage ring, and other possibilities.

Ideas for the world accelerator itself are not at all definite. Most often mentioned is the VBA, a proton accelerator (storage ring?) of more than 10 TeV. Part of the enthusiasm for such a project reflects the feeling that one of the major successes of CERN has been to demonstrate that many nations can work together to achieve a common goal, even one involving deeply integrated efforts in complex technology. It would indeed be a happy result if the same success could be achieved on a world wide scale, while providing the next generation of young physicists with completely untrodden territory to explore. □

New physics and new accelerators in Europe

rom B. H. Wiik

OVER the past few years great progress has been made in our understanding of the fundamental laws of nature as revealed by high energy physics. On the one hand, there has been an explosion of experimental discoveries—neutral weak interactions, probably charmed particles, perhaps a new lepton. Obtained at the present generation of high energy accelerators—CERN, DESY, FNAL and SLAC—most of these discoveries support the idea that weak and electromagnetic interactions can be unified in a single gauge theory. These experimental discoveries have been rivalled by a similar theoretical explosion. It now seems possible to suggest that the strong nuclear forces may also be reflections of an underlying gauge theory of quarks. It is even permissible to dream of the superunification of all elementary particle forces.

Experiment and theory in high energy physics must engage in a continuous dialogue for this progress to continue. Do new quarks exist beyond charm? Are there yet heavier leptons? What is the structure of the weak and the strong interaction? Is there a symmetry between quarks and leptons? Answers to some of these crucial questions may be forthcoming from experimental facilities which are now available or under construction. In Europe the hopes are centred on the ISR and the SPS at CERN and at DORIS and PETRA at DESY.

ISR

At the ISR—the large proton-colliding beams at CERN—a new generation of experiments specifically designed to search for new particles is under way and results are expected within a year. With impressive ease CERN recently commissioned

the SPS, a new 400 GeV proton synchrotron. The SPS supports, among other studies, extensive programmes in neutrino physics and high energy muon scattering. These lepton beams are well suited for the production of hadrons composed of new quarks and the experiments are also expected to give important clues to the nature of the strong interaction.

PETRA

The commission of PETRA, the high-energy e^+e^- accelerator now under construction at DESY, is little more than a year away. This storage ring will initially explore the energy range up to 30 GeV in centre-of-mass and ultimately probe even beyond 50 GeV. As has been proven with SPEAR at SLAC and DORIS at DESY, e^+e^- collisions are powerful sources of new particles, both quarks and leptons. At the highest PETRA energies the neutral

weak interaction may start to become comparable to purely electromagnetic effects.

Need for higher energies

However, there are crucial questions which can only be settled at energies well above those available with the present accelerators. From energies of a few keV to a few hundred GeV the weak interaction is well described by the point Fermi interaction resulting in a total cross section increasing linearly with energy. For the weak interaction to be consistent with unitarity, one generally assumes that it is mediated by the exchange of heavy vector bosons, to which gauge theories usually give masses between 50 GeV and 100 GeV. The simplest gauge model which relates the electromagnetic and the weak interaction predicts a charged $W^\pm$ boson with a mass around 65 GeV and a neutral Z^0 boson with a mass about 80 GeV. It is obviously crucial to find the intermediate vector bosons and to directly demonstrate that the electromagnetic and the weak interactions are simply manifestations of a single interaction. Another very important question which requires super-high energies to answer, is related to the strong interaction. If strong interactions are described by an asymptotically free gauge theory of quarks, then they should grow progressively weaker at very short distances. Short distances can only be probed at very high energies.

There have been several recent studies in Europe and elsewhere of accelerators with sufficiently high energies to confront these questions in the near future. At present, plans in Europe are centred on upgrading existing facilities at CERN. The boundary conditions are low cost and a reasonably short time-scale. Two projects which meet both these conditions take advantage of the excellent properties of the SPS to collide either antiprotons or electrons with the protons in the main ring.

Upgrading SPS

In the $p\text{-}\bar{p}$ option protons and antiprotons are injected into the SPS in opposite directions and made to collide in one or perhaps two of the long straight sections. The SPS can run continuously at 270 GeV resulting in a cms energy of 540 GeV for the $p\text{-}\bar{p}$ system. This option will provide a first glance at strong interactions in a new energy region where cosmic ray studies give tantalising indications of novel phenomena. It also offers the enticing possibility of directly producing the $W^\pm$ and Z^0 . Rates for these processes have been estimated using a Drell-Yan mechanism and found to be acceptable provided a luminosity of at least $10^{30} \text{ cm}^{-2} \text{ s}^{-1}$ can be reached. Recent results from the ISR on stochastic cooling and from Novosibirsk on electron

cooling—both used to increase the phase space density of the antiprotons—may make such luminosities within reach. A working group at CERN is now studying the following scheme which combines both methods. Protons from the 28 GeV proton synchrotron are used to produce antiprotons with momenta around 4 GeV/c. These are injected into a small storage ring, cooled stochastically, decelerated to 100 MeV kinetic energy and transferred to a second ring where they are further cooled by an intense electron beam travelling with the same velocity as the antiprotons. To accumulate a sufficient number of antiprotons this process is repeated many times over—in fact it takes nearly a day to accumulate the 10^{12} antiprotons needed. After accumulation, the antiprotons are transferred to the SPS and accelerated together with the protons to high energies. This is a very exciting project and a vigorous study is now under way to see if it can be realised with luminosities of $10^{30} \text{ cm}^{-2} \text{ s}^{-1}$ or above. A similar project is also under study at FNAL.

In the electron-proton option the proponents suggest injecting electrons from a small synchrotron like NINA into a large electron ring mounted in the SPS tunnel. Electrons with a maximum energy of 25 GeV to 30 GeV are made to collide with protons of energies from 140 GeV to 400 GeV during a normal or somewhat extended SPS cycle, with an intermediate flat top. Collisions can take place in two interaction regions and the peak luminosity may be of the order of $10^{32} \text{ cm}^{-2} \text{ s}^{-1}$. This scheme allows the normal SPS programme to continue and since the protons only remain for very short times in the SPS ring, all problems connected with storing a tightly bunched proton beam are minimised.

The proposed electron-proton option will extend the momentum transfer and energy range presently available with the neutrino and the muon beams at the SPS or at FNAL by nearly two orders of magnitude, probing a region where a point-like weak interaction would be stronger than the electromagnetic interaction. However, according to gauge theories the weak interaction will be damped such that both interactions will be of similar strength. The experiments will elucidate in detail how the weak and the electromagnetic interactions are unified—do only one $W^\pm$ and one Z^0 exist as presently conjectured, or is a more complicated mechanism involving many new heavy bosons responsible? Crucial information on the strong interaction can also be obtained by using virtual photons to probe the strong forces at short distances. Do they indeed weaken as the relevant distances shrink? Conclusive tests of this conjecture require momentum transfers well above those available at present accelerators.

Both the $p\text{-}\bar{p}$ and the $e\text{-}p$ option will

greatly advance our understanding of nature and they are both natural upgradings of the present SPS accelerator. Detailed studies of technical feasibility, and estimates of the cost and the interference with the SPS both during construction and operation are now under way for both projects, with the aim of making a full evaluation of both options by the end of 1977.

Future accelerators

Apart from exploiting existing facilities, one might also be optimistic and hope that Europe will be able to construct a large new accelerator within the next 10 years.

What questions might then be pressing, and what sort of large new accelerator would be needed to answer them? Probably we will want to study in detail the decays and interactions of the intermediate vector bosons. Do they have the characteristic gauge couplings apparently necessary for a calculable renormalisable theory? Does the debris of $W^\pm$ and Z^0 boson decays contain as yet unknown quarks or leptons? But by that time we may hope to be probing beyond gauge theories. In all such models yet constructed, the mass differences between hadrons and leptons, and between the photon and the vector bosons, arise by breaking the gauge symmetry with the Higgs mechanism. The associated point-like scalar Higgs particles are probably very difficult to find, but may be our only window into a world beyond the gauge principle. Perhaps also the superunification of the strong with the weak and electromagnetic interactions will be another crucial issue 10 to 15 years from now.

Many members of the European high energy community feel that these questions can best be answered by colliding electrons and positrons at very high energies. The results of first studies of such large e^+e^- rings were reviewed at a week-long working meeting organised by ECFA (European Committee for Future Accelerators) at DESY at the end of February this year (see accompanying article). This meeting created much enthusiasm among the participants and detailed feasibility studies of such an accelerator are now under way. Besides the arguments of pure physics, there are also strong political arguments for an e^+e^- ring as the next major European high energy project. These arguments are based on the fact that large proton synchrotrons and proton-proton colliding rings in the multi-TeV range are now under active consideration in both the US and the USSR. A European high energy e^+e^- ring would therefore be unique and fit well into attempts to coordinate the construction of new high energy accelerators on a world-wide basis. □

The accelerator after next

from D. J. Miller

"WHY have a conference about the accelerator after next, when we are just building the next one?" That is the question a lot of us were asking as we flew to Hamburg on 22 February for a 6-day working meeting on electron-positron storage rings of 100 GeV (the LEP, Large Electron Positron project). The organisers, ECFA—the European Committee for Future Accelerators—seemed to be looking a very long way into the future but a number of the participants argued that we should commit ourselves to building such a machine as soon as the money can be found.

The reason for their urgency lies in the present theoretical situation. Results from the present e^+e^- storage rings, from CERN and from Fermilab are giving increasingly firm and consistent support to the idea of charm and its relation to the neutral current in weak interactions. As a consequence, theorists are building more and more detailed models of elementary particles, and predicting quite specific properties for the forces which govern their interactions. The final unification of the weak, electromagnetic and strong forces has not been written down yet, but many parts of the unification scheme exist and will probably survive in something like their present form. In particular, a family of massive particles, the intermediate vector bosons ($W^\pm$ and Z^0), is required to account for the weakness of the weak interaction, and another family of 'Higgs bosons' is needed to relate the $W^\pm$ and Z^0 to the photon the carrier of the electromagnetic force. Precise predictions exist for the masses of the $W^\pm$ and Z^0 , though nobody claims that these predictions are unique. It is also expected that, as e^+e^- collision energies are increased, the weak interaction should grow in strength compared with the electromagnetic force. At the predicted mass of the Z^0 , 80 GeV/ c^2 —equivalent to 40 GeV on 40 GeV e^+e^- collisions—one of three things should happen. There could be a resonance, similar in some ways to the ψ and ψ' but even more interesting to analyse because its decays would tell us exactly how the weak neutral current interaction couples to every other kind of particle. There could be a steady increase of the weak interaction rate which would completely swamp the falling electromagnetic rate. Or there might be

a slow levelling-off of the increase in the weak interaction rate, with no resonance. Such a result would set a lot of puzzles, but their answer would come directly from a study of the events seen.

If the Z^0 resonance is seen, then it is expected that pair production of W^+ and W^- would also occur at a slightly higher energy, and at these energies it might also be possible to observe the production of Higgs bosons by way of $W^\pm$ or Z^0 intermediate states.

Some theorists are sufficiently confident in these predictions that they say we should go ahead as fast as possible and build the LEP machine. Others are more cautious. The first two 15 GeV on 15 GeV e^+e^- machines will not work until 1978 (PETRA in Hamburg) or 1979 (PEP in Stanford, California). The CERN SPS is just taking its first data. A large number of the predicted effects will begin to be seen at the 5 to 10% level with these devices. Perhaps we can get some more indirect evidence about the masses of the $W^\pm$ and Z^0 from neutrino interactions. Then there are relatively cheap advances which could be made by adding an antiproton-proton facility (270 GeV on 270 GeV) to the SPS, or by adding a 25 GeV electron ring to collide with 270 GeV SPS protons. Above all, money is tight and may be getting tighter. Who would pay for a new machine costing over one thousand million Swiss francs to build, and consuming over one hundred megawatts of power?

Electron machines are very greedy for power because the beams radiate energy away in the form of synchrotron radiation. That is why it is much easier to build high energy proton accelerators than electron accelerators. But electron-positron collisions seem to be a much better way of doing high energy physics. When protons collide at high energy the really interesting part of the interaction is between the constituent parts of each proton, the three quarks. This means that, on average, only one third of the energy of each beam is actually available in the basic quark-quark collision, compared with e^+e^- collisions where all the energy of each beam is concentrated in one particle. In addition, in proton-proton interactions, the quark-quark collision is accompanied by a splash of relatively uninteresting particles from the other two 'spectator' quarks in each

proton. The most important class of electron-positron collisions is much simpler to understand because the initial particles are completely annihilated, and the final state is produced in a well-defined way.

The ECFA working-meeting reviewed the theoretical and technical case for building an LEP machine. Possible detectors were discussed and it was clear that the cost of detectors for colliding-beam machines goes up with energy in a much gentler way than the cost of detectors for fixed-target machines such as the SPS. Perhaps the most vital discussions were on the feasibility and costs of the e^+e^- machine itself. Burt Richter from Stanford had made a preliminary investigation of the parameters of a 100 GeV on 100 GeV storage ring. He based his cost estimates on the PEP ring, but suggested that a much bigger machine might gain a considerable benefit from mass-production of components. Other accelerator designers checked his calculations and confirmed that the machine was certainly feasible, even without great advances in technology. One very desirable technical innovation was pointed out. Large numbers of radio-frequency cavity resonators are needed all round the machine to provide the accelerating field which replaces beam energy lost in synchrotron radiation. But half the energy supplied to these cavities does not go into the beam; it is dissipated by resistive losses in the cavity walls. If reliable superconducting cavities can be developed these losses could be almost eliminated. Small laboratory models have been built, but a major programme of research will be needed before production models of superconducting cavities can be designed.

The final opinion of the meeting was very positive. This is the next major accelerator project that should be proposed. It is probably not too big for Europe to build on her own, although collaboration from the USA or elsewhere might be desirable. It would give direct answers to the most important theoretical questions, whereas all other machines, existing or planned, will only give indirect answers. What must now be decided is how to match an LEP project to existing commitments and how to convince governments that the expenses will be worthwhile. □

THE presence of envelope glycoproteins of oncoviruses (the new name for oncornaviruses (Fenner *Virology* **71**, 371; 1976)) on the surface of normal cells has been known since the recognition of murine G₁X antigen and of chick helper factor in 1969. Since then there has been much discussion of whether the expression of endogenous viral antigens has a functional role in cellular differentiation. Certainly there is differential expression of viral antigens in different tissues and at different stages of development, but Strand, August and Jaenisch in the February issue of *Virology* (**76**, 886; 1977) conclude from studies of oncovirus gene expression in embryos of different inbred mouse strains that viral antigen synthesis is incidental to differentiation. While I agree that embryos of mice or chickens that show minimal endogenous viral gene expression appear to develop quite well, it is worth noting that feral mice and chickens (jungle fowl) do express viral antigens, so one can say at least that there is no natural selection against viral antigen synthesis. Furthermore, Chen and Vogt in the same issue of *Virology* (**76**, 740; 1977) find that oncovirus envelope glycoproteins are detectable in a variety of avian species.

In this week's *Nature*, (page 23) Elder, Jensen, Bryant and Lerner describe a more detailed study of the glycoproteins (gp 70) related to C-type oncoviruses in mice. By analysis of serotypes and of tryptic peptide maps, they can divide gp70 molecules associated with normal tissues into different groups, more or less related to gp70 specificities of virus isolates. Now most mouse strains inherit several distinct oncovirus genomes

Oncoviruses and cell membrane antigens

from Robin Weiss

and this may account for much of the polymorphism. For instance in strain 129 mice, the serum gp70 resembles that of xenotropic virus, whereas seminal fluid gp70 is more closely related to the FMR group of viruses, not previously thought to be endogenous. Some of the tissue gp70-like molecules may not be linked to oncovirus glycoproteins at all. We do not know whether oncovirus glycoproteins have evolved from cellular antigens or *vice versa*, although it is well known that oncoviruses readily recombine for this genetic marker.

If viral glycoproteins serve as differentiation markers on the surface of normal cells, one might also expect cellular membrane proteins to be assembled into virus envelopes. In fact, this has been evident since de Thé, Becker and Beard (*J. natn. Cancer Inst.* **32**, 201; 1964) demonstrated the presence of leukocyte membrane ATPase in the envelope of avian myeloblastosis virus. Indeed, Beard used this enzyme activity as a biochemical assay of virus particles. Aupoix, Huppert and Vigier (*C. r. hebdom. Acad. Sci. Paris* **282**, 1379; 1976) have demonstrated chick cellular antigens in Rous sarcoma virus particles which are inactivated by treatment with anti-chick serum plus complement, and Hecht and Summers (*J. Virol.* **19**, 833; 1976) have shown that vesicular stomatitis virus picks up H-2 antigens when grown in mouse cells. What is most intriguing, how-

ever, is the report by Bubbers and Lilly last month in *Nature* (**266**, 458; 1977) that Friend virus particles selectively incorporate H-2D^b antigen. It is interesting enough that one particular histocompatibility antigen is selectively assembled in the virus envelope but Bubbers and Lilly further point out that the H-2^b haplotype confers a relative resistance to Friend disease mediated by T-cell cytotoxicity against associative recognition of both viral and H-2^b antigens on the cell surface (Blank, Freedman & Lilly *Nature* **260**, 250; 1976). Thus the association of membrane antigens which act in Zinkernagel and Doherty's (*J. exp. Med.* **141**, 1427; 1975) 'altered-self' recognition system on the cell surface may also be reflected in the virus envelope. Lindenmann (*Biochim. biophys. Acta* **355**, 49; 1974) has argued for many years that viruses could be exploited as immunological adjuvants; complexes of viral and cellular glycoproteins in virus envelopes might be most potent immunogens.

The degree of selectivity of the assembly of H-2^b into Friend virus is a little surprising in view of the variety of unrelated glycoproteins that enveloped viruses can assemble. Perhaps this is most strikingly seen in phenotypic mixing between unrelated viruses, where the 'foreign' antigen can be detected by changes in the infectivity of the virus (Zavada *Methods in Biology*, **6**, 109; 1977). This is how Chen and Vogt (*op. cit.*) detected functional viral glycoproteins in various species of pheasant and quail. No less than seven other papers in the same issue of *Virology* report studies on the assembly and behaviour of glycoproteins in enveloped animal viruses.

Cathodoluminescence topography

from John Walker

DIAMONDS are reputed to look good in candlelight; to the scientist they look even better in electron light (cathodoluminescence—the emission of light during bombardment by electrons, as in a TV screen). Cathodoluminescence topography is a technique which has developed relatively recently and complements the older techniques of X-ray and UV topography. Scientists at the University of Bristol have recently published (Hanley, Kiflawi & Lang *Phil. Trans. R. Soc.* **284**, 329–368; 1977) their fascinating results on topo-

graphically - identifiable sources of cathodoluminescence in natural diamonds, including some fine colour photographs.

Their photographs carry a message, which as yet cannot be entirely deciphered. For example, a greenish-yellow luminescence system denoted H3, which is well-known from previous non-topographic studies, is observed at slip traces and dislocations and at 'platelets' (planar precipitates on {100} planes—see *Nature* **266**, 209; 1977). However, the usual method

of producing the H3 defect is to irradiate the diamond with electrons or neutrons and then anneal it. Now, Hanley *et al.*'s diamonds might have been irradiated by natural radioactivity—indeed some of them undoubtedly had been, as discussed below—but such damage is confined to a thin surface layer. Hence these H3 defects, lying deep within the crystal, must have been produced by other means.

It is known that the H3 defect is composed of a radiation damage product (probably a vacancy), trapped at

the 'A' form of nitrogen aggregate (probably a $\langle 111 \rangle$ -oriented substitutional nitrogen 'molecule'—see *Nature* **265**, 317; 1976). One can speculate that it could also be created without irradiation by slip in the same way as some EPR centres apparently are. In this process two adjacent $\{111\}$ planes of the crystal move relative to each other, and any unfortunate nitrogen molecules bridging the planes are sheared apart (Scherbakova *et al.* *Sov. Phys. Dokl.* **20**, 725; 1975).

Another puzzle is that H3 is not normally observed in type II diamond, whereas Hanley *et al.* detect the slip traces and the H3 luminescence in type II as well as type I regions in their specimens. One way in which these problems might be resolved is to deform plastically diamonds in the laboratory, and study their cathodoluminescence before and after.

Polarised H3 luminescence was observed from the platelets. Platelets were originally thought to be composed of nitrogen. Recent evidence suggests that they may be carbon in some interstitial arrangement, though there is still controversy on the matter, as Hanley *et al.* emphasise. On the carbon interstitial hypothesis one would have to assume that the H3 defects merely decorate the platelets rather than being an integral structural part of them, though in that case it is a little surprising that the luminescence is polarised. The uniformity of the luminescence along a platelet strongly suggests that they are an intrinsic part of the structure, and hence that the platelets do contain nitrogen. It is worth recalling that Lang's model for the platelets (*Proc. Phys. Soc.* **84**, 871; 1964) contain sets of $\langle 100 \rangle$ -oriented nitrogen 'molecules'; this similarity with the A centres may explain the H3-luminescing platelets.

Hanley *et al.* also observed infra-red emission from the platelets. Kiflawi and Lang (this issue of *Nature*, page 36) have confirmed this observation and have shown that it is highly polarised, and due to a broad-band system peaking at 1.25 eV, previously known to be connected with the platelets. This also seems to be an intrinsic platelet property rather than decoration by something else.

Other luminescent systems known to involve radiation damage were detected by Hanley *et al.* They included the H4, 3H (*sic*) and 575 nm systems, and were usually present in a thin surface layer whose thickness (30 μm) corresponded very well with the expected penetration depth of α particles. Hence they were probably created by natural radioactivity.

Hanley *et al.* draw attention to the intimate relationship between optical properties and growth features, and to

the inhomogeneity of most diamonds. They strongly recommend detailed examination of specimens, preferably by X-ray topography, before any experiments in which homogeneity matters. This has proved a pitfall in the past, particularly where correlation of different properties has been involved.

Since their measurements were done at or above room temperature, and largely in the visible spectral range 1.8 to 3.2 eV, extensions of the work are awaited with interest. Lower temperatures should bring greater sensitivity in the detection and identification of luminescence, and a wider spectral range might lead to further associations between point defects and growth features or other extended defects.

Diamond is the ideal subject for cathodoluminescence topography. It is transparent and shows a great many luminescence systems in the visible and near-visible spectrum. Furthermore, the processes by which it is synthesised, both industrial and natural, are not well understood; topographic studies give us some idea of how diamonds grew and what subsequently happened to them. Whether the technique can be applied as profitably to other materials is an open question. $\square$

How aseismic ridges behave

from Peter J. Smith

As newly produced oceanic lithosphere moves away from a spreading centre it cools and contracts. The result is that the average depth to the seafloor, which is $2,500 \pm 300$ m at active oceanic ridges, gradually increases with age until it is $5,800 \pm 300$ m for lithosphere formed during the late Jurassic-early Cretaceous. But although this general relationship between depth and age applies to most oceanic lithosphere, all the oceans contain many anomalously shallow features such as plateaus, rises and aseismic ridges. Such bodies have been relatively neglected by geologists, presumably because they appear not to form part of first-order seafloor spreading theory. Yet some information is available on aseismic ridges, not least from the Deep Sea Drilling Project (DSDP) which has now sampled at least 13 of them.

In an attempt to discover whether anything can be said about the origin and subsequent behaviour of such ridges, Detrick *et al.* (*Earth planet. Sci. Lett.* **34**, 185; 1977) have now brought

all the DSDP results together. And apparently something can be said. Consider, for example, the group of major aseismic ridges comprising Ninetyeast Ridge, Chagos-Laccadive Ridge, the southeast Mascarene Plateau (all Indian Ocean), Walvis Ridge and Rio Grande Rise (south Atlantic). DSDP cores indicate that all five contain older shallow-water sediments or subaerial material (ash, for example) and that the younger sediments become progressively more deep-water and more characteristic of deposition in an open marine environment. It would seem from the sedimentary record, therefore, that these ridges formed at or near sea level and have subsequently subsided by up to several thousand metres.

But by what mechanism did they subside? To find out (or perhaps to test a preformed hypothesis?) Detrick and his colleagues have determined, for each core which penetrated the volcanic basement, the age of the basement (usually from the palaeontological age of the basal sediments) and its depth corrected for the isostatic loading of the overlying sediments. When these ages and depths are plotted together, most of the points are found to lie in a curved band which bears a remarkable resemblance to the curve of age against the depth to be expected on the assumption that ridges subside at the same rate as normal oceanic crust. The agreement is not exact, for in addition to the scatter of points there is a tendency for the measured subsidence to be a few hundred metres less than that predicted. Nevertheless, it is clear that all five ridges have subsided at a rate comparable to that of normal oceanic crust.

That being the case, Detrick *et al.* propose a simple model in which an aseismic ridge forms at a spreading centre by excessive volcanism which builds the ridges up to sea level. By analogy with large-scale shield volcanism, the construction of the ridge is taken to be fairly rapid (perhaps 0.5–1.5 Myr) and the isostatic adjustment of the crust to the new ridge is assumed to occur almost simultaneously with the volcanism. Then all that happens is that the spreading, subsiding lithosphere carries the ridge along and down with it. In other words, the subsidence of an aseismic ridge is a purely thermal effect associated with the cooling and contraction of the lithosphere upon which the ridge is built.

Of course, real ridges do not often conform exactly with the model. At formation they may lie slightly above or below sea level; and since their tops are seldom if ever perfectly horizontal planes, there will be variations in level even within a single ridge. Moreover, a ridge may form slightly off the spreading axis, giving it an age rather younger

than that of the surrounding crust; and even a small deviation from the axis can produce a significant effect here because the depth-age curve close to a spreading centre is steep. Then for a ridge formed subaerially, the amount of subsidence occurring before the ridge top sank below sea level will not be recorded in the measured subsidence. The net result of all such effects will be to produce a scatter of points along the depth-age curve and also to bias the points towards too small a measured subsidence.

In the circumstances, then, it is perhaps surprising that the difference between the measured subsidence and the subsidence predicted from the behaviour of normal crust is generally no more than 300–400 m. However, such relatively small deviations apply only to the five ridges under consideration; other aseismic ridges conform much less well. For example, the Iceland–Faroe Ridge evidently formed above sea level and remained that way for at least 15 Myr before beginning its subsidence. On the other hand, Coiba Ridge, Carnegie Ridge and Cocos Ridge (Pacific) seem to have formed well below sea level and are thus now unusually deep. Then again, Madagascar Ridge, Broken Ridge and the Naturaliste Plateau (Indian Ocean) seem to have complicated histories of subsidence and uplift.

In view of these exceptions, it seems that the Detrick model can make no claim to widespread application. Yet there is more to it than that. It could be said, at least as an hypothesis, that the model applies generally to all aseismic ridges that form at spreading centres and at sea level. It does not necessarily follow from this, however, that all aseismic ridges are formed at spreading centres and at sea level. What may be true nevertheless is that, wherever a ridge forms, when it subsides it does so at a rate comparable to that of normal oceanic crust and as a direct result of the cooling and contraction of the lithosphere. □

New twists in bacterial flagella

from Michael Spencer

SINCE the last comment in these columns (*Nature*, 263, 370; 1976) more light has been shed on a number of problems relating to the structure of bacterial flagella. The first is the geometrical puzzle set by bacteria having more than one flagellum: if all are helices undergoing real or apparent rotation, can they avoid tangling together? It is a simple matter to test

this empirically by twiddling two pieces of wire between the finger and thumb, but a rigorous analysis is more daunting. Macnab (*Proc. natn. Acad. Sci. U.S.A.* 74, 221; 1977) has now tackled the problem using geometrical and hydrodynamic theory, assisted by working models.

His conclusions confirm the finger-and-thumb analysis, showing that if a group of left-handed helices starts to rotate in a counterclockwise sense when viewed looking towards the basal points of attachment, the flagella are constrained to rotate in phase with each other and will continue to do so without hindrance; they do, however, undergo some distortion so as to form a compact bundle. This is what is observed in the normal swimming of bacteria. When the direction of rotation reverses, however, the helices should rapidly interlock so as to prevent further rotation (unless the flagella are very short, as occurs in some species). If both the handedness of the helix and the sense of rotation are reversed, jamming is not expected and normal forward motion can be resumed, with the flagella pointing to the rear.

Now comes the surprising part. Macnab and Ornston (*J. molec. Biol.* 112, 1; 1977) report that a bacterium does not simply stop when the flagella reverse their rotation: the bundle flies apart, and the clockwise-rotating flagella are observed to have the smaller pitch characteristic of the 'curly' mutant. As first shown by Shimada *et al.* (*Nature* 254, 332; 1975), this has a right-handed helix. If the process is slowed down by irradiating with blue light in a high-viscosity medium these flagella sometimes form a curly bundle, giving forward motion again. Normally, however, there is a brief period of 'tumbling' followed by reversion to the previous state: a bundle of left-handed helices is reformed and the organism swims off in a new direction.

This periodic change of direction is an essential part of the chemotactic response, for external stimuli seem to influence the probability of tumbling: movement in a favourable direction suppresses the urge to tumble. This applies whether the bacteria are moving towards an attractant or away from a repellent, and must involve some kind of memory mechanism linked to the receptors (Macnab & Koshland *J. molec. Biol.* 84, 399; 1974). The observed change of helix parameters seems to be essential, for without it the bacteria might simply stop and start again without changing direction, and there would be no point in reversing the sense of rotation. Only in certain special cases can backward motion be

sustained.

The obvious question is "What makes the helix change when rotation is reversed?" After studying a number of mutant forms and the effects of abnormal growth media, Macnab and Ornston conclude that the torsional stress arising from a reversal of rotation actually induces a change in the packing of the flagellar subunits, so that the structure flips from a left-handed to a right-handed helix. On removal of the stress, the structure clicks back into its normal shape. Their paper includes a number of photographs in which the transition is caught propagating out from the base of the flagellum, where torsion is expected to be greatest. As confirmation of this idea, abnormal curly flagella are observed to flip into the normal form under stress—the reverse of the normal process.

An outstanding problem—apart from the key question of how rotation is generated—is the nature of the forces that hold the subunits of a flagellum together. Kamiya and Asakura (*J. molec. Biol.* 108, 513; 1977) have some new evidence which supports the idea that electrostatic charge interactions are important. It has been known for some time that reconstituted flagella from *Salmonella* can change from the left-handed normal helix to the right-handed curly form on reduction of the pH. Kamiya and Asakura now report that the same transition can be induced by an increase of pH above neutrality. In this case, as for the acidic transition, the curly form is attained by passing through an intermediate ('coiled') form. It can hardly be a coincidence that this sequence of transitions is just what would be expected from a geometrical packing model of the kind suggested by Calladine (*J. theor. Biol.* 57, 469; 1976). It looks as if the ionisation induced by extremes of pH causes longitudinal rows of subunits to rearrange themselves, causing a discrete change in the helix parameters for each row thus transformed. Calladine's working hypothesis of a 'hinge' between subunits, giving two alternative arrangements, is supported by several biological examples; the latest to be described is tomato bushy stunt virus (Winkler *et al.* *Nature*, 265, 509; 1977).

This latest work on flagella illustrates how electrostatic and mechanical forces can separately induce a similar structural change. Macnab and Ornston make the point that a stress-induced transition may be relevant in other systems having regularly-arranged subunits. Who knows, at present, whether it might not be at the heart of muscular contraction? But that is another story. □

Wanted alive—but handle with care

PHYSIOLOGY laboratories in Britain, Germany and the United States are urgently seeking suppliers of the tropical giant water bugs of the genus *Lethocerus*, whose flight muscle is one of the most important preparations used in the study of the molecular mechanisms of contraction in insect flight muscle.

During a symposium held recently in Oxford, UK, the problem of obtaining living material from tropical countries and the lack of success in breeding these bugs in the laboratory were discussed. The laboratories shown in the table are all now in the market for the living insects; the table shows approximate requirements and acceptable species. They would be glad to hear from anyone who can provide these insects and would be willing to make contracts with reliable suppliers. Transit costs would be refunded and all prices are quoted in US dollars.

These giant bugs are aquatic but are easily collected when they fly to lights in damp weather—in the rainy season now approaching in many of the countries where they are found. Provided they are individually restrained in envelopes with an adequate air supply, up to 50 can be packed in a perforated tin and sent by air freight. These insects have a poisonous bite and should be handled with care.

The proceedings of the symposium will be published by Elsevier-North Holland Biomedical Press later this year, entitled *Insect Flight Muscle*.

Address	Regular monthly requirement	Maximum requirement for irregular supply	Acceptable species
Professor J. W. S. Pringle, ARC Unit, Dept of Zoology, Oxford OX1 3PS, UK	20 at \$5 each	3,000 at \$0.5 each	A + B
Dr D. C. S. White, Dept of Biology, The University, York, UK	4 at \$5 each	120 at \$0.5 each	A + B + C
Professor J. C. Rüegg, Universität Heidelberg, II. Physiologisches Institut, Im Neuenheimer Feld 326, 6900 Heidelberg, FRG	1 at \$10 each	2,000 at \$0.5 each	A
Dr K. C. Holmes, Max-Planck-Institut für Medizinische Forschung, 6900 Heidelberg, Jahnstrasse 29, FRG	2 at \$10 each	250 at \$2 each	A
Dr M. K. Reedy, Dept of Anatomy, Duke University Medical Center, Durham, North Carolina 27710, USA		Special arrangements	
Dr J. Squire, Biopolymer Group Dept of Metallurgy and Materials Science, Imperial College London SW7 2BP, UK		Special arrangements	
Dr G. Steiger, Los Angeles County Heart Lab., Center for Health Sciences, UCLA, California 90024, USA		Special arrangements	

A = *L. maximus* (Venezuela, Trinidad); *L. indicus* (India, Malaysia, Indonesia); *L. colossicus* (Mexico).
 B = *L. cordofanus* (Africa, South East Europe); *L. annulipes* (Guyana, West Indies); *L. insulanus* (Australasia); *L. griseus* (Florida, Mexico).
 C = *L. uhleri* (South-East United States).



Lethocerus: \$5,000 market



A hundred years ago

M. HENRY GIFFARD is constructing, near the Champ de Mars at Paris, a workshop for the preparation of sulphate of iron. The apparatus was tried for the first time last Friday, when the balloon *Eole* was inflated in an hour and a half, and was sent up with an aéronaut. The capacity of the balloon being 220 cubic metres, the rate of production is very satisfactory. It is expected that the sale of sulphate will cover almost all the expenses, so that numerous scientific ascents may be made in the ensuing summer. The monster captive balloon of 20,000 cubic metres will be inflated by the same process.

from *Nature* 16, 3 May, 17; 1877.

articles

Evidence of Precambrian continent–continent collision in Western Norway

Erling J. Krogh

Mineralogisk-Geologisk Museum, Sars'gate 1, Oslo 5, Norway

Norwegian gneiss eclogites are formed in situ within the temperature–pressure range 300–800°C, 8–22 kbar. The regional K_D^{ga+cpx} distribution pattern indicates an increasing P–T trend towards the coastline. Retrograde reactions indicate a nearly isothermal uplift history. A plate tectonic model, involving continent–continent collision and subduction is proposed for the formation of these eclogites.

ECLOGITES commonly occur as bands and lenses within the West Norwegian gneiss region. The surrounding rocks are mica-rich schists, biotite–plagioclase gneisses and biotite–two–feldspar gneisses^{1–4}. They are assumed to be metamorphic equivalents of original supracrustal rocks. The metamorphism of these gneisses lies in the almandine amphibolite facies, but eclogites are also found within a complex of granulite facies rocks⁵.

K–Ar ages on hornblendes from the eclogites give 1,750–1,850 and 980–1,170 Myr, and micas give 370–415 Myr (ref. 5). A three point K–Ar isochron based on data from McDougall and Green⁶ (sample no. 756) gives ~940 Myr (W. L. Griffin, personal communication). Rb/Sr whole rock datings on the surrounding gneisses give ~1,700 Myr (refs 4, 6) and ~1,000 Myr (ref. 7), while mineral Rb/Sr (ref. 7) and U/Pb (ref. 8) data indicate a Caledonian effect. These datings suggest that the eclogites are of the same age, and have the same crustal history as the surrounding gneisses.

The formation of Norwegian eclogites has, however, long been a controversial subject. O'Hara and Mercy⁹ suggest that the eclogites were emplaced into the gneisses as wedges of crystalline rocks that were derived from the upper mantle. Lappin¹⁰ believes that the eclogites were brought to near-surface conditions by an upwelling mantle (for example a mid oceanic ridge), and taken along with a moving oceanic lithosphere plate to a subduction zone. As the subducted lithosphere went downwards, the eclogites were mechanically mixed in with the overlying continental material, where we find them today. The main argument for a mantle origin of the eclogites is that the P–T conditions necessary for eclogite formation are not realised within the crust^{9,10}. Lappin¹⁰ further argues that the maximum pressure during the metamorphism of the gneisses did not exceed 10 kb, a "limiting pressure assigned to amphibolite facies by various authorities".

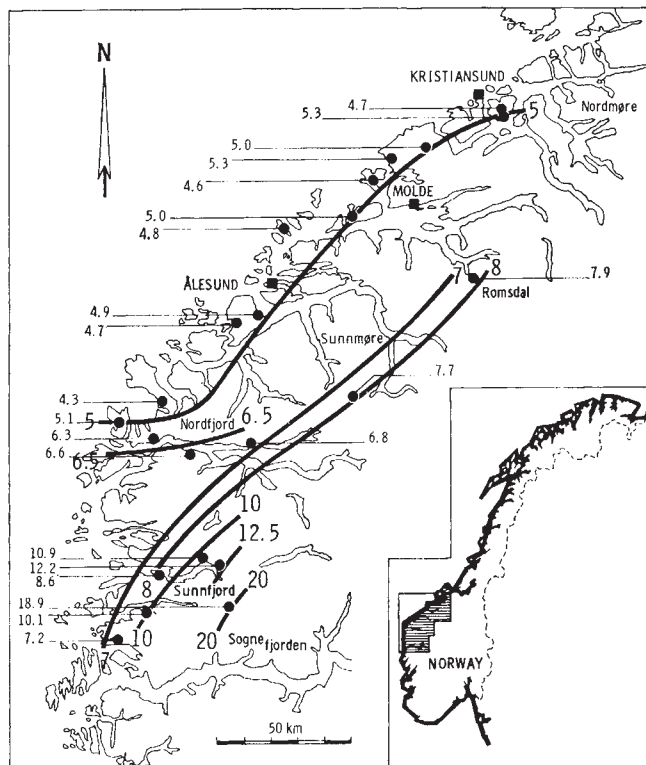
Bryhni² argues from field and structural data that although some of the eclogitic bodies are tectonic inclusions, they have not been transported over long distances relative to the enclosing gneiss. Most of the eclogites, however, form concordant lenses or bands parallel to the foliation of the gneiss. There is structural and petrological evidence for the formation of eclogites *in situ* together with the enclosing gneisses^{3,4,11–14}. Griffin and Råheim¹² describe dolerites that are partly altered through coronites to eclogites, and show that they were formed *in situ* at the same P–T conditions as the eclogites in the same

area. This implies that eclogites may be formed within the crust.

I have recently discovered blueschist facies mineralogy in eclogites from the Sunnfjord area, also indicating a crustal origin of these rocks.

Samples from more than 30 different localities (Fig. 1) were chosen for a detailed study of the Norwegian gneiss eclogites. The general primary mineral assemblage in the eclogites from Sunnfjord area is garnet + omphacite ± phengite ± paragonite + barroisite/glaucophane + quartz + rutile ± kyanite ± zoisite. In the Nordfjord, Sunnmøre and Nordmøre areas, the general primary mineral assemblage is garnet + omphacite + amphibole ± phengite ± quartz + rutile ± kyanite ± zoisite. In these areas orthopyroxene bearing eclogites are also fairly common. The primary mineral assemblage in these rocks is garnet + clinopyroxene + orthopyroxene ± amphibole ± quartz + rutile. Primary plagioclase is not present in any of the examined samples, except from albite inclusions in garnet in

Fig. 1 Locations of analysed ga+cpx pairs from eclogites, with minimum K_D^{ga+cpx} values. The iso- K_D -lines show a regular pattern, with K_D decreasing towards the coastline. Some of the given K_D values are mean values from several small bodies within the same area.



some few cases. Secondary plagioclase is, however, observed both together with secondary orthopyroxene as a breakdown product of quartz + garnet, and as symplectitic intergrowths with secondary diopside or hornblende. These secondary features are most common in the NW part of the area.

Experimental work has shown that the distribution of Fe^{2+} and Mg between garnet and a co-existing FeMg-mineral is a function of temperature and pressure¹³. If the distribution coefficient is defined as

$$K_D = \frac{(\text{FeO/MgO})^{\text{ga}}}{(\text{FeO/MgO})^{\text{FeMg-min}}}$$

then the K_D value at constant P will decrease with increasing temperature, and vice versa. Similarly, the Al_2O_3 content of orthopyroxene coexisting with garnet has also shown to be a function of T and P (refs 15–19). At constant P the Al_2O_3 content in orthopyroxene will increase with increasing T .

To calculate pressures and temperatures of formation for the eclogites, EMP analyses of coexisting ferromagnesian minerals were done. Special attention was paid to the chemical zoning of the minerals.

Ferric iron was calculated assuming stoichiometry. This method seems to give very consistent results, and EMP analyses of five samples from one eclogite body in Sunnfjord give a variation in $K_D^{\text{ga+cpx}}_{\text{D(FeO/MgO)}}$ of 12.6 to 13.4. Assuming an analytical error in SiO_2 of $\pm 1\%$ by weight, this will result in a maximum variation in K_D in one of these samples of 10.9 to 13.8, which corresponds to a temperature difference of only 40 °C.

The methods used for determination of P and T are those worked out by Wood¹⁶ (Al_2O_3 in opx), Råheim and Green¹³ ($K_D^{\text{ga+cpx}}_{\text{D(FeO/MgO)}}$), and Krogh and Råheim ($K_D^{\text{ga+ph}}_{\text{D(FeO/MgO)}}$; work in preparation). The presence of either ga+cpx+ph or ga+cpx+opx will thus allow simultaneous solutions of two equations to give unique values of T and P . If both phengite and orthopyroxene are missing, the $K_D^{\text{ga+cpx}}$ (ref. 14) can be combined with experimental and empirical work on the jadeite content of clinopyroxene^{20,21} to give P - T estimates. This will, however, only give minimum values of both P and T since there is no co-existing plagioclase to saturate the clinopyroxene with regard to jadeite.

The P - T dependance of $K_D^{\text{ga+FeMg-min}}$ and the Al_2O_3 content in orthopyroxene coexisting with garnet, can also be used to interpret the chemical zoning of co-existing minerals

with regard to changing P - T conditions. In almost all the eclogites examined here, zoning of the ferromagnesian minerals is a common feature. The zoning is most prominent in the garnets, but zoning is also observed in the other ferromagnesian minerals. The most common type of zoning, which occur in the Sunnfjord and parts of the Nordfjord area, is that in which the FeO/MgO ratio in clinopyroxene and phengite increases from core to rim, and the same ratio in the coexisting garnet, and thus the $K_D^{\text{ga+FeMg-min}}_{\text{D(FeO/MgO)}}$ decreases in the same direction. In the coexisting orthopyroxene, the Al_2O_3 content increases slightly from core to rim. According to experimental studies this reflects an increase in both T and P , from core to rim. The reversed zoning is observed in the NW parts of the gneiss region (Fig. 1), except that the Al_2O_3 content in orthopyroxene increases strongly from core to rim. This zoning reflects a decrease in both T and P . This reversed zoning is also observed in the outermost rim of normal zoned minerals. In metamorphic rocks, however, only the rims of adjacent grains can be said to represent equilibrium conditions. Whether or not cores of coexisting minerals were in equilibrium with each other at an early stage of the metamorphism is often hard to evaluate. In the cases where, for example, clinopyroxene occurs as inclusions in the garnet core, this equilibrium seems to be obvious. In these cases $K_D^{\text{ga+cpx}}_{\text{D(core)}}$ is constant, regardless of whether one uses analyses of cores of separate grains, or analyses of the inclusions and the adjacent garnet core. In these rocks $K_D^{\text{ga+cpx}}_{\text{D(core)}}$ should give P - T conditions appropriate to an earlier stage of the metamorphism. A good example of this, taken from an eclogite with prograde zoned minerals, is as follows (sample IB12, Nordfjord):

$$K_D^{\text{ga+cpx}}_{\text{D(core)}} = 8.6; K_D^{\text{ga+cpx}}_{\text{D(incl)}} = 8.6; K_D^{\text{ga+cpx}}_{\text{D(rim)}} = 6.8$$

This very clearly shows that the $K_D^{\text{ga+cpx}}_{\text{D(incl)}}$, which is taken close to the garnet core, gives the same K_D as the $K_D^{\text{ga+cpx}}_{\text{D(core)}}$, while $K_D^{\text{ga+cpx}}_{\text{D(rim)}}$ of adjacent grains gives the lowest value. The Jd -content of the clinopyroxene is rather constant (43%), and this gives the following temperatures and pressures: core and inclusion: 645 °C, 15.0 kbar; rim: 700 °C, 16.0 kbar. This suggests that the minerals grew during an increase in both P and T , that is, prograde metamorphism. In other cases, however, amphibole and/or phengite, rather than clinopyroxene, occur as inclusions in the garnet core. The garnet cores were thus not obviously in equilibrium with a clinopyroxene, but clearly coexisted with amphibole and/or phengite. This is commonly the case in the southernmost part of the area (Fig. 1). Therefore one should not use $K_D^{\text{ga+cpx}}_{\text{D(core)}}$ in such cases, but rather $K_D^{\text{ga+ph}}_{\text{D(core)}}$. A more detailed discussion of this problem is given elsewhere (E. J. K. and Råheim, in preparation).

The calculated P - T conditions define a path of metamorphic evolution for the Norwegian gneiss eclogites (Fig. 2). The prograde part of this curve extends from ~ 8 kbar, 300 °C to ~ 22 kbar, 800 °C. This is consistent with the trend for formation of eclogites given by Råheim and Green²².

From about 800 °C there is a drop in pressure and temperature which defines the retrograde trend. From Fig. 2 it is further evident that normal zoning occurs up to 700–750 °C. Above that temperature reversed zoning occurs.

The normal zoning of the minerals is interpreted as the result of crystal growth during prograde metamorphism; the inner parts of the mineral grains failed to re-equilibrate during the growth^{14,22}. The normal zoning could thus be denoted prograde zoning.

At a certain temperature, the diffusion rates will be high enough to homogenise the minerals, and thus wipe out the prograde zoning. By cooling and uplift, the rims of adjacent grains will continue to re-equilibrate by diffusion until the system again reaches the actual threshold temperature for diffusion. Good evidence for this is recorded in the normal (prograde) zoned minerals that show a thin zone of reversed (retrograde) zoning at the rims. Råheim and Green²² also describe these features, and they reach the same conclusions.

Fig. 2 Pressure and temperature calculations, based on co-existing mineral pairs from West Norwegian gneiss eclogites, define a prograde trend from ~ 300 °C, 8 kbar to ~ 800 °C, 22 kbar (I), and a retrograde trend from ~ 800 °C, 22 kbar to ~ 700 °C, 5 kbar (II). 1, 2, Melting curves for muscovite granite with excess H_2O , and dry muscovite granite, respectively²⁵. 3, Albite $\rightleftharpoons$ jadeite + quartz²⁰. 4, Eclogite $\rightleftharpoons$ granulite transition²³. 5, 6, 7, Kyanite/andalusite: andalusite/sillimanite and kyanite/sillimanite transitions, respectively^{26,27}.

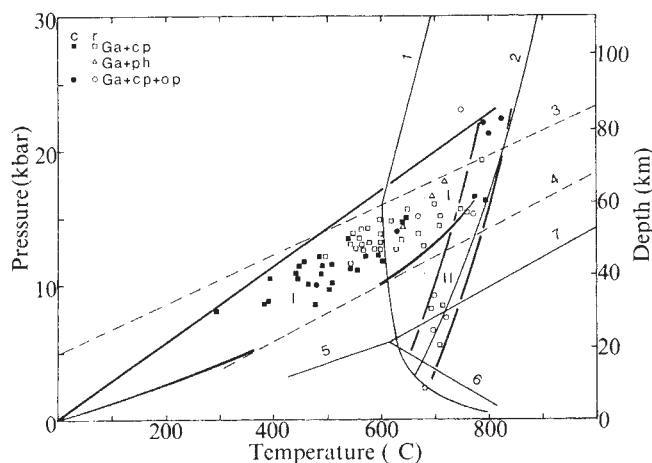


Table 1 Mineral zoning (K_D^{g+cpx}) and calculated pressure–temperature conditions in orthopyroxene-free and orthopyroxene-bearing eclogites

	Nordfjord, prograde		Sunnmøre, retrograde	
	Opx-bearing	Opx-free	Opx-bearing	Opx-free
$K_D^{(core)}$	19.0	15.7	5.0	5.0
$P : T^{(core)}$	10.2 kbar, 480 °C	9.6 kbar, 505 °C	22.3 kbar, 825 °C	19.3 kbar, 790 °C
$K_D^{(rim)}$	6.8	6.6	5.2	—
$P : T^{(rim)}$	23.0 kbar, 750 °C	17.6 kbar, 720 °C	6.6 kbar, 700 °C	—

The appearance of the secondary granulite facies assemblage orthopyroxene + plagioclase in the rocks showing retrograde zoning reflects the retrograde trend entering the granulite facies stability field²³ (Fig. 2). The very strong symplectitisation of primary omphacite in these rocks also suggests strongly decreasing pressure. Mysen and Griffin²⁴, however, give a more detailed discussion of this problem. The regional minimum K_D^{g+cpx} (Fig. 1), and thus the P – T pattern (Fig. 2) indicates an increase in maximum P and T from south to north-west.

It is also of interest to point out the similarities in mineral zoning, K_D^{g+cpx} , and thus the calculated P – T conditions, between orthopyroxene-bearing and orthopyroxene-free eclogites from the same areas. This is consistent both in the prograde and in the retrograde areas (Table 1).

Bearing in mind that the Jd -contents of clinopyroxenes in these eclogites only give minimum P – T values, both the zoning of the minerals (recorded in K_D variation from core to rim), and the minimum K_D values strongly indicate the same metamorphic history for these two types of eclogites.

The P – T path shown in Fig. 2 is consistent with that expected for regional metamorphic terranes now exposed at the surface; first a prograde (burial and heating) and then a retrograde (uplift and cooling) history. It is also evident from Fig. 2 that the rocks from a relative high dP/dT were exposed to a lower dP/dT environment. Thus there must have been a considerable change in the regional geothermal gradient in the area during the metamorphic cycle.

During subduction of a cold slab, the isotherms will be depressed. Once the subduction ceases, and during the succeeding uplift, the anomalous isothermal pattern will be smoothed out, and the area restored to its normal geothermal gradient. The subduction + uplift process can thus explain the metamorphic cycle that is reflected in the zoning of the minerals of the west Norwegian eclogites.

As mentioned above, datings suggest that eclogites are parts of a continental mass metamorphosed 1,700 Myr BP, and later disturbed at ~1,000 and ~400 Myr BP. The convergent margins in this case must therefore have been of a continent–continent collision type, followed by a subduction of one of the continents under the other. Before a continent–continent collision, a subduction of oceanic crust under one of the continents would be expected, thus producing an Andes type orogenic belt. This will cause an increase in the thickness of the continental crust at the continental margin. The continental–

continental collision itself will also lead to a further thickening of the continental crusts. By a further subduction of one of the continents on the cold side of an Andes type mountain chain, the subducted continental slab may be exposed to depths of 80–100 km without significant melting (Fig. 2). This type of orogene-forming process will also lead to a high degree of isostatic disequilibrium. This implies a rapid isostatic uplift, high reliefs and a rapid erosion rate, that is, a relatively rapid decrease in pressure.

The present data give good evidence to suggesting that plate tectonics were active as early as 1,850–1,700 Myr ago. Evidence for the existence of an early (> 1,850 Myr ago) proto-Atlantic ocean of the Pacific type is also found further north along the same coastal margin in the Lofoten–Vesterålen area (E. J. K., in preparation).

I conclude that all the eclogites examined here can represent crustal rocks metamorphosed *in situ* during subduction of a continental crust to depths of 65–80 km. At these depths some mantle material may have been picked up, but the separation and recognition of the mantle derived rocks is not straightforward at present.

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Conformational change theory for auxin structure–activity relationships

T. M. Kaethner

Biophysics Unit, ARC Institute of Animal Physiology, Babraham, Cambridge, UK

An hypothesis is advanced that the hormonal activity attributable to the heterogeneous group of plant growth compounds called auxins is a direct result of the ability of the bound auxin to undergo a simultaneous conformational change or reorientation with the receptor, while in the binding site.

THE structure of the plant growth hormone indol-3yl-acetic acid (IAA, natural auxin) was established in 1934¹, and considerable effort has been devoted to elucidation of molecular requirements regarding auxin structure and hormonal activity^{2–6}, but as yet no theory has predicted comprehensively the relationship between molecular structure and hormonal activity.

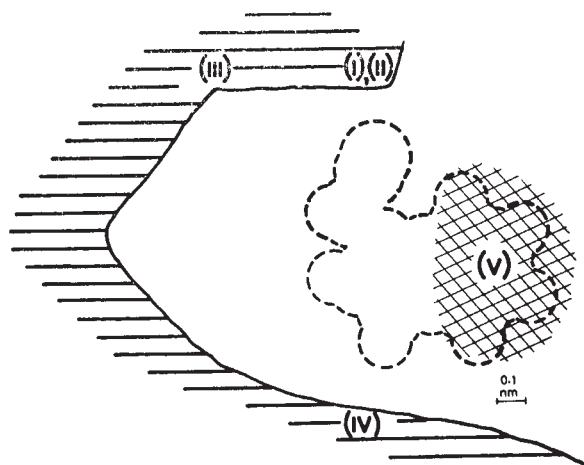


Fig. 1 Cross-sectional representation of the proposed IAA-receptor site, showing the outline of IAA (broken lines) bound in the recognition conformation. Two electropositive regions (i) and (ii) are bisected by the plane of the section; region (iii) is electro-positive, whereas (iv) is electronegative. The cross-hatching for region (v) represents areas of hydrophobic interaction with one or both sides of the receptor cleft, which has proportions resembling those of a canopied crib.

In the hypothesis described here, the hormonal activity of the heterogeneous group of compounds called auxins⁷ is attributed to the ability of the bound ligand to undergo a simultaneous conformational change or reorientation with its receptor while in the binding site of that receptor. I propose that there is a single site for primary auxin action, possessing five regions of major discriminatory significance with regard to auxin binding (Fig. 1): (i) and (ii) are two electropositive regions, possibly paired, and are responsible for recognition of the carboxyl or other acidic group of the auxin; they also show the essential conformational change relative to the ligand and remainder of the receptor; (iii), a third adjacent electropositive region; (iv), an electronegative region on, or comprising the 'floor' of, the receptor site, normally responsible for hydrogen-bonding with the pyrrole nitrogen region of IAA; and (v) an hydrophobic cleft, interacting chiefly with the benzene ring region of the indole nucleus, rather than the entire ring. Thus the site has proportions resembling a canopied crib, three electropositive regions comprising the canopy, a fourth electronegative region delimiting the 'floor', with the hydrophobic regions as the sides. The site is open for auxin recognition and binding at the 'top' and 'front'.

Recognition of IAA by the electropositive regions (i) and (ii), and subsequent binding in the site, occur with the side chain of IAA in the plane of the ring such that the oxygens of the carboxyl group lie astride the ring plane, directed over the hydrogen of carbon 4 of the indole ring; this is termed the recognition conformation (Fig. 2). The bound IAA molecule now undergoes a simultaneous conformational change with the receptor; while still interacting with regions (i) and (ii) of the site, the carboxyl group and side chain of IAA assume a position perpendicular to the plane of the indole ring, such that the oxygens of the carboxyl group are aligned so as to be co-linear with an imaginary line joining carbon 3 and nitrogen of the indole nucleus; this is termed the modulation conformation (Fig. 2).

Thus, competence as an auxin requires adoption of the essentially planar recognition conformation, and an ability to move in concert with the receptor to the modulation conformation. The obvious corollary is that molecules which can bind in the site, but which are unable to show a simultaneous conformational change with the receptor, will not

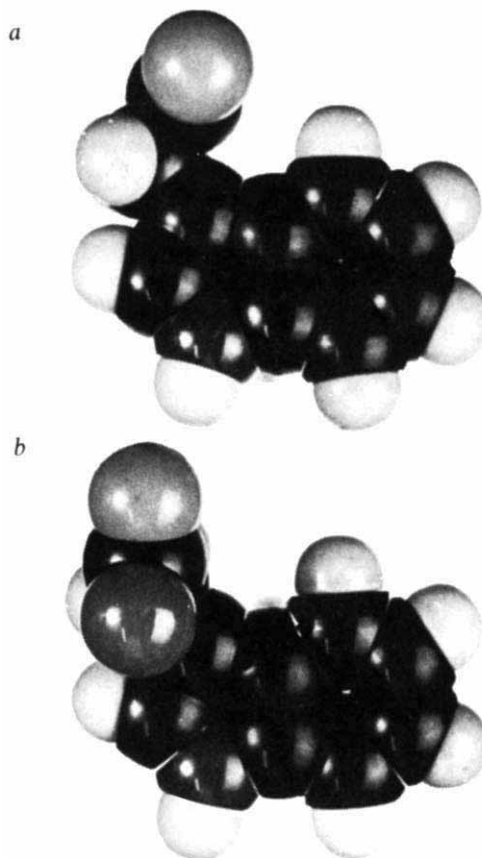
be active as auxins. By occupying the site ineffectually, such compounds will antagonise auxin action; the more potent auxin antagonists are proposed to have these properties of high affinity, zero capacity auxins.

To relate structural analogues of IAA back to this model, primary emphasis must be placed on the orientation of the side chain and carboxyl group of the test compound. These must be aligned so that their angle of inclination coincides as nearly as possible with that of IAA in its recognition conformation. Figures 3 and 5 illustrate this. With this reference point established, molecular comparisons among the whole family of auxins can be made, according to the simplistic approach that the role of a synthetic auxin is to reproduce as best as possible the features of IAA, especially its dimensions.

The exceptional molecular structure of auxins of the arylcarboxylic acid type necessitates elaborated discussion of their structure-activity relationships, but the activities of other synthetic auxins (including dithiocarbamates and acyclic analogues) can be assessed largely in terms of three factors: (1) hindrance to side chain mobility resulting from *ortho* substitutions about the side chain; (2) hindrance to side chain mobility resulting from substitutions on the side chain, and (3) hindrance to binding due to substituents in positions corresponding to substitutions on C₇, C_{7a}, or N₁ of IAA.

Factors (1) and (2) relate to the ability of auxins to show the required conformational change, that is their capacity to function; factor (1) is best exemplified by the influence of di-*ortho* substitutions in phenoxycetic acid derivatives, where hormonal activity is markedly reduced⁸⁻¹⁰, or lost⁹⁻¹¹

Fig. 2 IAA, with side chain in the recognition conformation (a). Oxygens are astride the ring plane. In the modulation conformation (b) the side chain moves to a position perpendicular to the ring plane, and the carboxyl group realigns as shown.



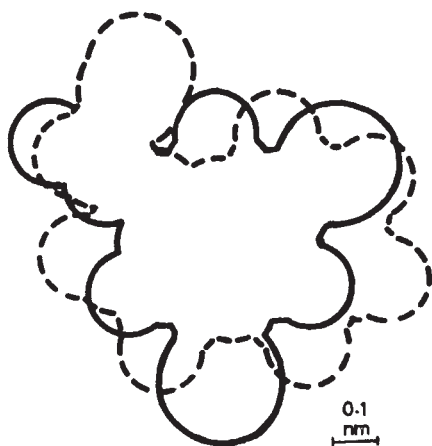


Fig. 3 Comparison of the molecular dimensions of IAA (broken lines) and 3,5-dichlorophenoxyacetic acid (solid lines), both in the recognition conformation.

unless the size of one of the *ortho* substituents does not exceed that of fluorine¹⁰. The greatly diminished activities for 2-methyl-IAA¹²⁻¹⁴, 8-chloronaphth-1-yl-acetic acid^{15,16} and 1,3-dichloronaphth-2-yloxy-acetic acid¹⁷, by comparison with their unsubstituted parent acids, further illustrate the point. The variable behaviour of naphth-1-yloxy-acetic acid in different bioassays⁴ is also explained on the basis of the rotational degeneracy of the side chain; extensive side chain movement is impeded by the *ortho* hydrogen at carbon 2 of the ring, so that any capacity for hormonal activity may be reduced to zero, with antagonistic properties arising^{18,19}, according to the degree of movement demanded by the receptor site in question. The same does not apply to the structural isomer naphth-2-yloxy-acetic acid, an auxin of moderate activity^{8,17-19}; the relocation of its side chain on the ring leaves the required movement sterically unhindered.

Factor (2) relates especially to optical isomerism in auxins. Two compounds differing only in their arrangement of groups around an asymmetric carbon in the side chain can have profoundly different activities as auxins. Not surprisingly, the absolute configuration of such enantiomers and their respective hormonal activities are very closely correlated; in all but a very few instances (discussed in detail in ref. 20) enantiomers with the D-configuration possess higher auxin activity than the corresponding L-forms, while the L-forms are often inactive as auxins or are auxin antagonists⁹.

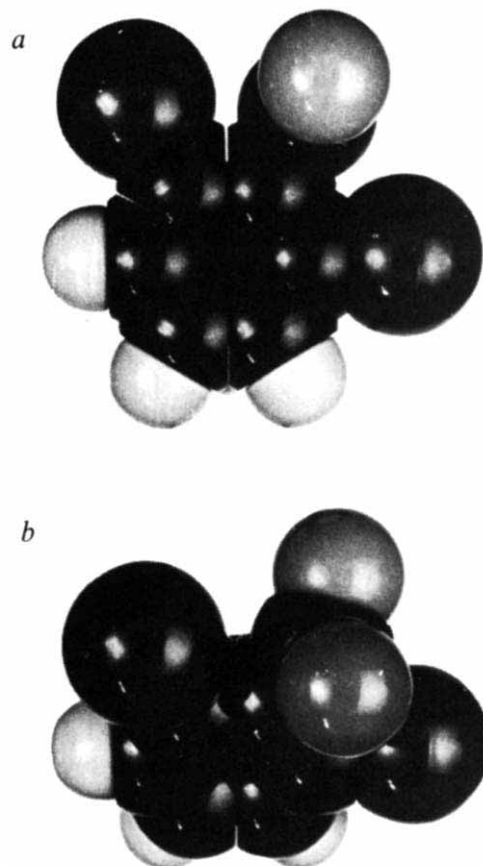
I propose that the basis of the differing properties of enantiomers is a single fundamental feature—the need to retain the ability to undergo a conformational change if capacity as an auxin is to be retained. Adsorption into the receptor site immediately imparts a sidedness on the auxin molecule, hitherto non-existent in most cases. Because the conformational change of the receptor is specifically directional, that of the ligand must be also, and movement of the carboxyl group to the face of the indole ring as shown in Fig. 2 is proposed as the only side chain movement able to confer hormonal activity. The ability of the side chain to move to the 'back' face (as depicted) is of no consequence to activity. Thus the contrasting steric locations of substituents in enantiomer pairs and the consequent effects on the ability of the side chain to move between recognition and modulation conformations is reflected in the general trends relating absolute configuration and hormonal activity. Side chain substituents in the L-configuration introduce intra-molecular steric hindrance to side chain movement, reducing hormonal activity and often imparting antagonistic properties. Generally the stereo-relationships imposed by the absolute D-configuration prohibit such intramolecular

effects. Methyl substituents on the side chain of auxins in the D-configuration may, however, reduce unfavourable electrostatic interactions with the electropositive region (iii) of the receptor site, resulting in increased affinity between ligand and receptor in the case of weak auxins⁹.

Data tabulated for the activities of all mono-, di- and tri-chlorophenoxyacetic acids¹¹ not only illustrate factor (1), but (3) also. All compounds displaying zero activity in the particular bioassays used by Leaper and Bishop possess either di-*ortho* substitution, or are unable, by face reversal (rotation about the bond linking the side chain to ring), to avoid placing a substituent in locations analogous to those which would be adopted in the site by substituents on C₇, C_{7a} or N₁ of IAA (di-*meta* phenoxyacetic acid derivatives, Fig 3); the reduced activities of 7-chloro-IAA¹³, N₁-methyl-IAA^{12,13}, 4-chloro- and 5-chloro-naphth-2-yloxyacetic acids¹⁷, relative to the unsubstituted parent acids, is also explained on the basis that the substitutions introduce novel interactions with the 'floor' of the receptor site, thereby reducing binding affinity.

Auxins of the arylcarboxylic acid type lack a spacing atom between the carboxyl group and ring structure, so that a conformational change of the type proposed for other auxins is not possible. Instead, arylcarboxylic auxins are proposed to show a molecular reorientation in conjunction with the conformational change of the receptor. As usual, recognition and binding occur with the oxygens of the carboxyl group astride the ring plane. Once in the site the entire molecule is envisaged to tilt through approximately 45° relative to the ring plane at recognition 'conformation', about an horizontal axis passing through ring

Fig. 4 2,6-dichlorobenzoic acid, with the alignment of the carboxyl group determining molecular orientation for the recognition 'conformation' (top). A modulation 'conformation' (below) equivalent to that adopted by IAA is assumed by partial rotation of the molecule about a horizontal axis passing through the centre of the ring.



carbons 2 and 5; in this manner the molecule assumes an equivalent modulation conformation (Fig. 4). This theory would endorse the two salient features of Veldstra's conclusions²¹ concerning benzoic acid structure-activity relationships: a non-flat structure, with the carboxyl group oxygens astride the ring plane, is essential for binding; all other requirements with respect to substituents, including the di-*ortho* substitutions responsible for the non-flat structure, are suggestive that absence of intermolecular hindrances to binding in the receptor site is critical, rather than a requirement for the presence of specific unsubstituted locations on the ring for binding.

There is insufficient evidence to make further conclusions about structural requirements for activity in benzoic acid derivatives. The observation that *para* substituents on benzoic acid exceeding the size of fluorine are incompatible with activity²¹ would seem to require the proviso that consideration also be given to possible stabilisation of charged resonance forms of the ligand. Thus the inactivity of *para* chlorinated benzoic acids²¹ and data on their potency as antagonists of auxin action²²⁻²⁴ suggest that such derivatives are accepted into the site, but are unable to show a re-orientation in concert with the receptor conformation change; presumably steric interaction between the *para* chloro group and the 'floor' of the site prevents the required reorientation from occurring. The *modus operandi* of 4-amino-3,5,6-trichloropicolinic acid as a hormone will resemble that of the benzoic acids, yet despite the presence of a chloro substituent *para* to the carboxyl group, this compound can show hormonal activity exceeding that of IAA²⁵. It is thought that resonance stabilisation of charges could influence the activity of this compound in two relevant ways; first, molecular dimensions may be altered to render it sufficiently different from *para* chlorinated benzoic acids; and second, the positively charged amino group is able to assume a location in the binding site analogous with that of the pyrrole nitrogen of the indole ring of IAA, so that favourable interaction with the proposed electronegative region (iv) might reduce hindrance to the '*para*' chlorine substituent during the molecular re-orientation required for activity.

Figure 5 illustrates an empirical method of approximately summarising the maximum ligand dimensions permissible for binding under the canopy region of the site. The expected variation amongst receptor sites (tissue-specific and species-specific) will prevent adoption of a single critical dimension universally applicable to auxins for the distance between 'top' of the ligand carboxyl group (in the recognition conformation) and the lowest molecular extremity. The values chosen for Fig. 5, however, exclude such diverse inactive auxin analogues as *trans*-cinnamic acid²⁶, 2,6-dibromo-, 2,6-diiodo- and 2,6-dimethoxy-benzoic acids³, 9-anthracenecarboxylic acid²⁷ and indol-3-yl-carboxylic acid^{2,12}.

True antagonism to auxin action (as opposed to general metabolic inhibition) is considered, on this hypothesis, to be due to two general types of compounds. One category can be regarded as high affinity, low capacity auxins, as they occupy the site but are unable to show a conformational change or reorientation with the receptor. Examples of this type are L(-)- α -(2-methyl-4-chlorophenoxy)-propionic acid^{28,29}, 4-chloro- and 2,4-dichlorobenzoic acid^{23,24}, 2,6-dichlorophenoxyacetic acid³⁰, and auxins with *isobutyric* acid side chains⁵. A second class of antagonists have the properties of low affinity, high capacity auxins; although side chain flexibility leaves such molecules with the capacity to function as auxins, the nature of the remainder of the molecule reduces affinity for the receptor site and these antagonists compete with auxins for receptor regions (i) and (ii). Examples of this second type are 3,5-dichlorophenoxyacetic acid, and phenoxyacetic acid and its 2-halogen derivatives⁵. Åberg's discussion of antagonism of potent

auxin action by weak auxins would be viewed as this second form of antagonism. Gradations between these two classes of antagonists will occur.

The hormonal activities attributable to L(-)- α -(indol-3-yl)-propionic acid³¹⁻³³, α -(indol-3-yl)-isobutyric acid^{8,33}, and L(-)- α -(benzothien-3-yl)-propionic acid³⁴ remain unexplained by this theory. Molecular models of these compounds suggest their side chain movement to the modulation conformation is not possible, and the theory would predict antagonistic properties for them; indeed, this is the case for α -(indol-3-yl)-isobutyric acid³⁵ and L(-)- α -(benzothien-3-yl)-propionic acid³⁴ in some bioassays. High auxin activity and strong antagonism, however, are viewed as mutually exclusive features, and it is not without precedent to comment that these compounds deserve more rigorous investigation^{3,6}. All structures closely resemble that of the natural auxin IAA, and the contradictory results presently available for them may reflect metabolic conversions to active forms within some test tissues.

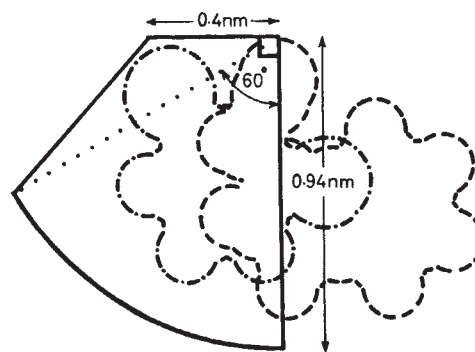


Fig. 5 An empirical method of approximately summarising the maximum ligand dimensions permissible for binding under the 'canopy' region of the receptor site (solid lines). Measurements are taken from the top of the carboxyl group when side chain (if present) and carboxyl group, in the recognition conformation, are aligned so as to coincide with the corresponding regions of IAA in its recognition conformation (IAA -----; 2,6-dichlorobenzoic acid - - - - -).

When this conformational change model for auxin action is discussed within the context of former theories of auxin structure-activity relationships, it is seen that it represents a rationalisation and extension of previous concepts. First, molecular significance can be attached to the five structural features characterising the many synthetic auxins known by 1938². A ring system provides the planarity to fit the cleft, an hydrophobic core for interactions with appropriate regions on the receptor site, and in the case of IAA, the molecular complexity to facilitate hydrogen bonding through the indole nitrogen region. Double bonds in the nucleus (or minimally, one double bond with the side chain attached to it³⁶) permit adoption of a recognition conformation with the side chain in the plane of the ring and a modulation conformation with the side chain perpendicular to the plane of the ring. A side chain arising from the ring nucleus provides the molecular flexibility required for simultaneous conformational changes with the receptor. A carboxyl group (or other acidic function) on the side chain is essential for auxin recognition by sites (i) and (ii); the stipulation that the carboxyl group be separated by one atom from the ring again relates to the need for molecular flexibility if hormonal activity is to be retained. The observation that a particular space relation between carboxyl group and ring was required for activity reflects the necessity for side chain conformational degeneracy so that the carboxyl group can move between two specific spatial locations defined by the extent of movement of the recognition

regions (i) and (ii) of the receptor as this shows its conformational change.

Furthermore, the proposed recognition conformation apparently coincides with the active auxin form suggested by Jönsson⁴, while the modulation conformation is equivalent to the active form proposed by Veldstra³. The premises of the two-point attachment theory³⁷ and three-point attachment and α -hydrogen theory³⁸ are encompassed by the hypothesis that side chain conformational changes are essential for auxin activity. The 5.5 Å (0.55 nm) charge separation theory³⁹ is a rigid hypothesis expressing the possible relevance of charged regions of auxins to receptor site binding; apart from the specific case of IAA, and the general requirement for an acid group on the ligand, it has little in common with this theory.

That auxin action entails more than a simple binding at a site has been intimated by others. Veldstra³ considered that auxins should possess "a capacity to cause a specific physiological response when fixed to the receptor (in dynamic equilibrium with free molecules!)". Similarly, discussing the influence of some alkyl side chain substituents on hormonal activity, Fredga and Åberg⁵ concluded that "the large substituent in some way hinders the side chain of the molecule from assuming the position leading to auxin activity". Unwittingly, both comments predict this hypothesis, that capacity to function as an auxin requires an ability to undergo a simultaneous conformational change or reorientation with the receptor.

An understanding of the biochemical significance of the proposed conformational change induced in the receptor by auxin binding must await characterisation of the site of auxin action. The nature of this conformational change theory of auxin structure-activity relationships is, however, compatible with current theories on the biochemical basis of auxin action, and not exclusively applicable to one only.

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Polymorphism of the major envelope glycoprotein (gp70) of murine C-type viruses: virion associated and differentiation antigens encoded by a multi-gene family

J. H. Elder, F. C. Jensen, M. L. Bryant & R. A. Lerner

Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California 92037

Structural comparison of the major envelope glycoproteins (gp70) from 35 different murine type C viruses and free gp70 expressed at various anatomical sites in the mouse showed that the gp70s are polymorphic products of a large multi-gene family encoding viral and differentiation antigens. Different proviruses are expressed in cells following distinct pathways of differentiation. When the various gp70s are grouped according to primary structure they fall naturally into viral host range classes, confirming the suspicion that C-type viral tropism is largely determined by the nature of the gp70 product expressed.

POLYMORPHISM of certain viral proteins is well known and often forms the basis for the serological classification and subdivision of

antigenically related viruses¹⁻³. Viral polymorphism probably reflects the response of specific viral genes to selective pressure, and as for the influenza viruses, may influence the dynamics of the host-parasite relationship³. When, however, one considers the murine C-type viruses, the issue of polymorphism takes on a unique significance in that these agents may spend at least part of their life cycle as proviruses integrated into the host chromosomes where they may be inherited according to mendelian expectations⁴⁻⁶. One or more of the proviral genes may be expressed at certain sites of differentiation, often in the absence of complete virus production⁶⁻⁹. Insofar as expression of the provirus is regulated during cellular differentiation, its gene products may be considered among the differentiation antigens of the mouse¹⁰. There are two important questions regarding polymorphism of C-type viral proteins as structural components of intact virus and cellular differentiation markers. The first question concerns whether the gene products are 'virus-like' with extensive divergence or more

'host-like', where even the products of the most polymorphic loci are relatively conserved. Second, we need to know whether different proviruses are expressed at different sites of differentiation, resulting in the presence of multiple subtypes of related viral proteins within a single animal.

Of the various gene products of endogenous C-type viruses, the major envelope glycoprotein gp70 is well suited for a study of polymorphism. This glycoprotein has a role in the host range, interference and neutralisation properties of the virus and carries the type-, group- and interspecies determinants which form the basis for viral subclassification. But a classification based on the functional properties of viral gp70 does not allow comparison at the molecular level and cannot be used in the absence of infectious viruses. Therefore, a more rigorous approach to the study of

Tryptic peptides of gp69, gp70 and gp45 are identical

Initial experiments were performed to determine the value of ^{125}I tryptic peptide analysis as a tool for structural comparison of related gp70s. One issue we needed to resolve concerned the possible presence of more than one gp70 in a given virus preparation, as was suggested by the early finding that Rauscher gp70 could be resolved into discrete bands with molecular sizes of 69,000 and 71,000 (refs 11, 12). If gp69 and gp71 were different gene products this would obviously complicate a structural study of polymorphism. In addition, we studied a protein with molecular weight of 45,000 which is precipitable by anti-gp70 antisera and is found in certain virus preparations. In Fig. 1 the tryptic peptide

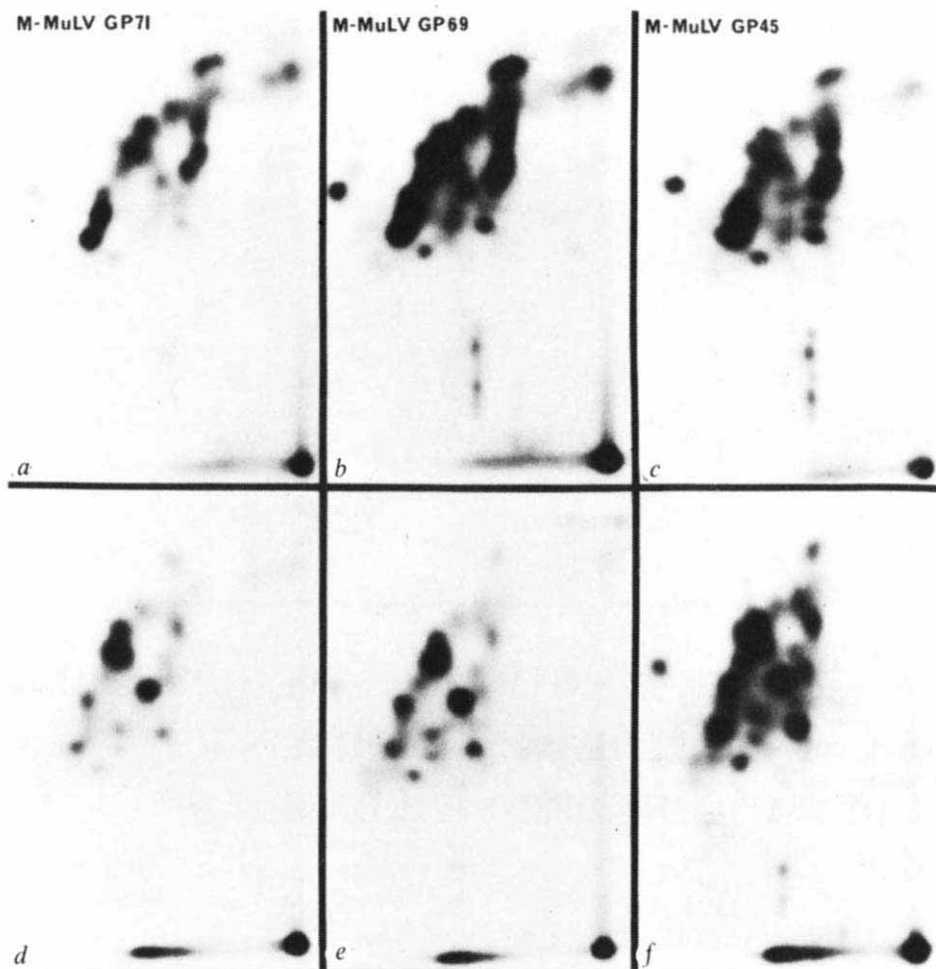
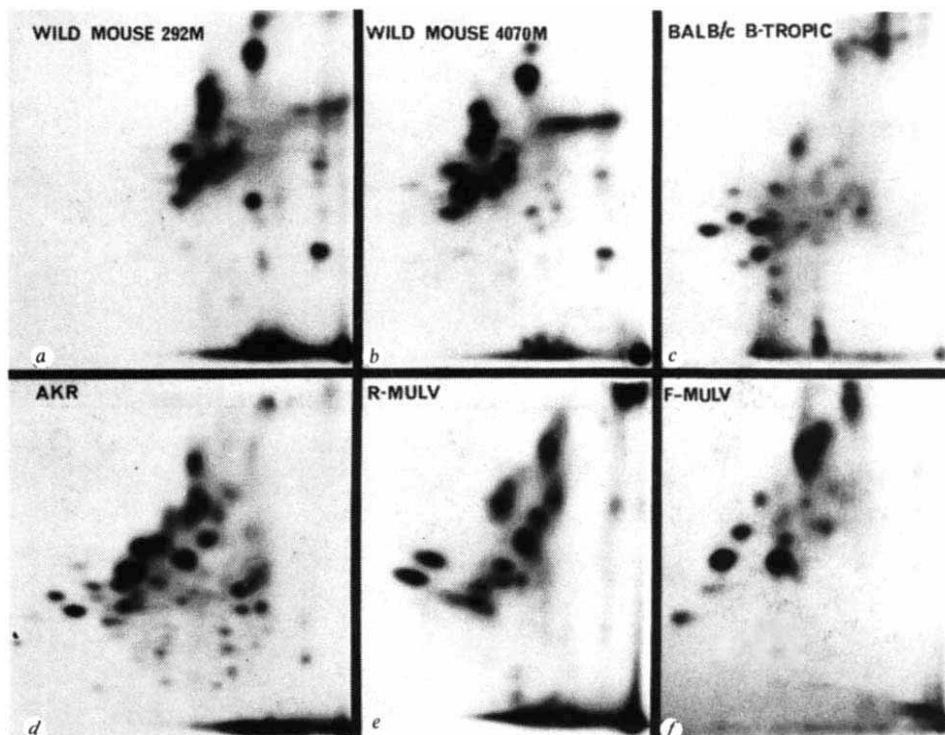


Fig. 1 Comparison of the tyrosine-containing tryptic peptides of Moloney virus gp71, gp69 and gp45. Moloney virus gp69/71 was purified by phosphocellulose chromatography and was a gift from Dr S. Kennel of Oak Ridge Laboratories. The protein (10 μg) was radioiodinated with ^{125}I (Amersham, specific activity about 15 $\text{mCi } \mu\text{g}^{-1}$) to high specific activity by the chloramine T method¹³ in the presence of either 0.5% NP40 in 0.5 M phosphate buffer (*a-c*) or 1% SDS in 8 M urea (*d-f*). Gp71, gp69 and gp45 were then separated by SDS-PAGE¹⁴, the gels were sliced and the slices containing the radioactive proteins were pooled and placed in 10% acetic acid. The slices were washed extensively with 10% methanol to remove SDS, dried with a heat lamp, and placed in a solution of 50 $\mu\text{g ml}^{-1}$ trypsin (Worthington, 27 IU mg^{-1}) in 0.05 M NH_4HCO_3 buffer (pH 8.0). The slices were incubated overnight at 37 °C, after which the solution was removed and lyophilised. The tryptic digests were then analysed on two-dimensional peptide maps¹⁵. The samples were dissolved in 20 μl buffer I (acetic acid-formic acid-water, 15:5:80) and 2–5 μl was spotted on to 10 cm $\times$ 10 cm cellulose-coated TLC plates (EM Laboratories). The samples were then electrophoresed at 1 kV for approx. 30 min, dried and chromatographed in a second plane using buffer II (butanol-pyridine-acetic acid-water, 32:5:25:5:20). The plates were then dried and analysed by autoradiography using Kodak RP-X-ray film. *a-c*, gp71, 69 and 45, respectively, radioiodinated in NP40; *d-f*, GP71, 69 and 45 radioiodinated in the presence of 1% SDS and 8 M urea.

polymorphism requires the use of techniques allowing direct comparison of primary structure. We report our comparison of the primary structure of 27 different virion-associated gp70 molecules and gp70 isolated from the sera and seminal fluid of nine different strains of mice. Based on the tyrosine-containing tryptic peptides of these gp70s, the C-type viruses of murine origin can be divided into several groups with varied degrees of relatedness. Polymorphism exists within each of these groups. Compared to viruses recently isolated from inbred or from feral mouse populations, the gp70s of the laboratory-derived murine viruses vary extensively in structure. The structure of the gp70 found free in the serum of all mice tested is relatively conserved and seems to be the product of the same or similar xenotropic virus gene regardless of how many other proviruses the mouse may harbour. The provirus coding for the serum protein is distinct from that coding for the genital tract gp70 and thus unique proviruses are expressed at different sites of differentiation.

analyses of gp71, gp69 and gp45 present in a preparation of purified Moloney virus gp70 are shown. Figure 1*a-c* shows that the tyrosine-containing peptides of these three proteins are identical. Another question answered in this experiment is the extent to which the carbohydrate portion of the molecule influences the resultant tryptic map. It is estimated that 32% of the mass of gp70 is carbohydrate, whereas carbohydrate is only 6–9% of gp45¹⁶. Because the tryptic maps of gp69, gp71 and gp45 are identical, it is apparent that the mass of carbohydrate does not greatly influence our analyses and this is because the glycopeptides do not migrate in the apolar solvent used here for chromatography (our unpublished results). Another methodological question concerned the extent to which conformation of the molecule influenced radioiodination and the subsequent peptide map. Figure 1*a-c* shows the tryptic peptides of the proteins iodinated in the presence of NP40 (0.5%) and 0.05 M sodium phosphate buffer; in Fig. 1*d-e* these proteins are radioiodinated in the presence of 1% SDS and 8 M urea.

Fig. 2 Comparison of the tyrosine-containing tryptic peptides of gp70 isolated from several endogenous and exogenous ecotropic (mouse-tropic) viruses. *a*, Wild mouse 292M; *b*, wild mouse 4070M; *c*, BALB/c B-tropic; *d*, AKR; *e*, R-MuLV; *f*, F-MuLV. Rauscher virus, produced in chronically infected JLSV-9 cells, was obtained from Dr Jack Gruber of the Resources branch of the NCI. SC-1 cells producing AKR virus (AKV-1) were obtained from Drs W. Rowe and J. Hartley and virus pools were prepared from the third and fourth subculture in our laboratory. Friend virus¹⁷ was a gift of Dr W. Parks. B-tropic virus (no. 138) was isolated from a 3-methylcholanthrene-induced tumour of a BALB/c mouse. The no. 138 virus is completely restricted in FV-1¹⁸ cells even at high multiplicity of infection. Pools were prepared from the third to fifth tissue culture passage for use in this study. The origin and clonal purification of wild mouse ecotropic viruses (1504M, 292M, 4070M, 1740M) have been described¹⁸. Enriched fractions of gp70 were obtained from virus isolates by immunoaffinity chromatography as described¹⁹ with several modifications. Monospecific goat antisera to Rauscher virus gp70 (G230) was used as the source of antibody. Proteins were eluted from the affinity column with a 0–2 M TCA gradient at neutral pH as suggested²⁰. The majority of non-specific protein came off the column from 0 to 0.75 M TCA. Gp70 was eluted from the column at 1–1.5 M TCA. After dialysis against phosphate-buffered saline, gp70 from all isolates retained antigenicity and remained stable for several months at –20 °C. After dialysis, the gp70 fractions were radioiodinated as in Fig. 1 and direct precipitates were performed with G230 antiserum. The immune precipitates were then electrophoresed on SDS-PAGE and treated for peptide mapping as described in Fig. 1.



Changes in the intensity of certain spots have occurred, but the total pattern is the same. This suggests that conformation of the gp70 molecule may influence the extent of iodination of certain peptides but all peptides are labelled even under relatively mild conditions.

The virion-associated gp70s can be divided into groups

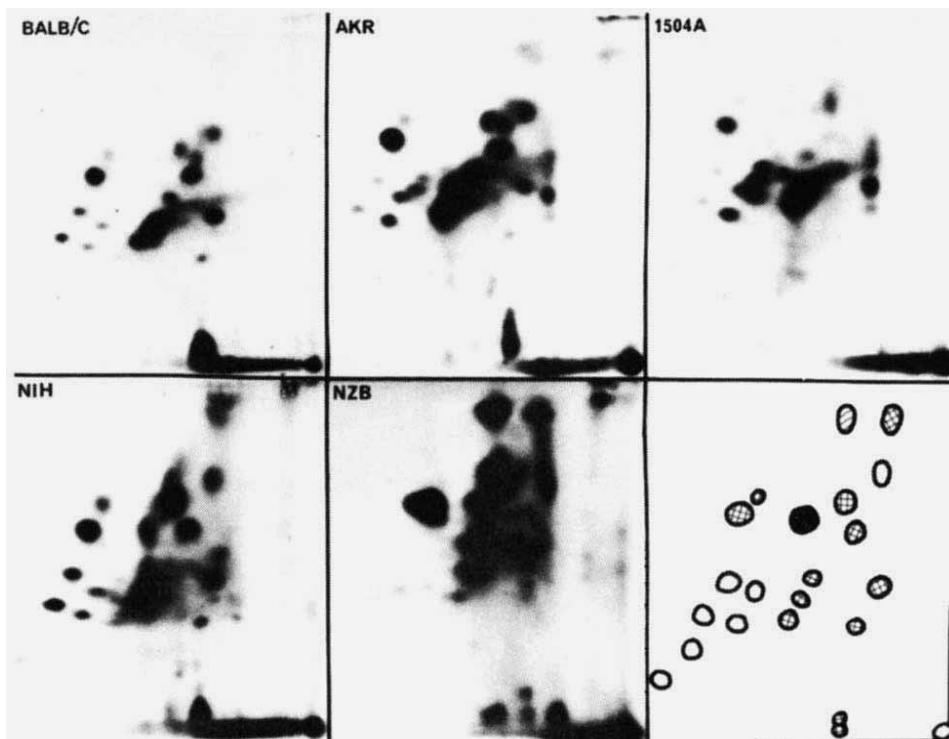
Based on tryptic peptide analyses of 27 virion-associated gp70s, the following conclusions are drawn: (1) The gp70s of the murine viruses which both grow in and infect mouse cells (ecotropic viruses) can be divided into several divergent groups. This point is illustrated in Fig. 2, in which the tryptic peptides of 6 endogenous and exogenous ecotropic viruses are shown. Overall, the gp70s of the various wild-type ecotropic (*a–d*) viruses are more related to each other than to the FMR (*e, f*, Fig. 1*a*) viruses. The gp70s of the ecotropic viruses of wild mice (Fig. 2*a, b*) form a closely related group, distinct in structure from the prototype AKR viruses (*d*). The gp70s of FMR viruses, Friend, (Fig. 2*f*), Rauscher (Fig. 2*e*) and Moloney (Fig. 1) are divergent from each other and the other ecotropic viruses, possibly as the result of a recombinant origin. These data indicate that extensive diversity exists from one group to another in spite of the fact that ecotropic gp70s share the common property of facilitating entry of their respective viruses into mouse cells, but not cells of other species. (2) The gp70s of the endogenous viruses which are produced by mouse cells, but can only infect cells of other species (xenotropic viruses), form two groups, one of which is closely related to the gp70s of viruses which can infect both mouse cells and cells of other species (amphotropic). In Fig. 3, the tryptic peptides of several xenotropic virus gp70s and an amphotropic virus gp70 are compared. BALB/c, AKR and NIH xenotropic gp70s are closely related to one another and to the amphotropic gp70, but are distinct from the NZB xenotropic virus gp70. We interpret the differences within the large group of xenotropic gp70s (as well as the gp70s of C₃H and NZW, not shown) as polymorphism within a group. The gp70 of the NZB xenotropic virus is divergent to the point that we place it along with (NZB × NZW) F₁ (not shown) into a separate group of xeno-

tropic virus. These findings are in general agreement with serological studies of others²³. (3) The gp70s of amphotropic wild mouse viruses form a single highly conserved group closely related to one of the xenotropic groups (Fig. 3). The structure of the amphotropic gp70s will be completely described in a separate report (Bryant *et al.*, in preparation).

To determine whether the cell in which the viruses were propagated influenced our results, we compared the structure of the gp70s of three different amphotropic viruses, each of which had been grown in mouse, rabbit and mink cells. The gp70s were identical irrespective of in which cells the viruses were grown. This suggests that host cell modification of gp70 is not a significant variable in our studies.

The serum gp70 of laboratory and wild mice is homologous to the NZB xenotropic virus gp70

Large amounts of gp70 are present in the serum of many strains of mice in the absence of intact viruses⁷. To determine which provirus(es) encode serum gp70 we compared the tryptic peptides of gp70 isolated from the serum of several strains of mice. In Fig. 4, the tryptic peptides of virion-associated NZB gp70 (*a*) and 5 gp70s isolated from sera of different murine strains (*b–f*) are shown. The data indicate that the serum gp70s are members of a highly conserved group, all of which are closely related to the NZB xenotropic virus gp70. Additionally, NZW and two wild mouse serum gp70s had the same structure (data not shown). Thus, the serum gp70 of all mice we have tested is probably a partial expression of a single provirus, which is expressed as complete virus only in the NZB and (NZB × NZW)F₁ mice. In so far as that part of the provirus coding for serum gp70 is concerned, there has been little divergence and it appears to behave like a moderately polymorphic murine gene. These structural studies support the data of Stephenson and Aaronson in which it was shown that serologically the serum gp70 resembled that of the NZB xenotropic virus²³.



viruses, but different interference and neutralisation specificities and are negative in the XC test^{18,21,22}. BALB/c (no. 435) and NIH Swiss (no. 436) xenotropic viruses were a gift of Drs Hino, Stephenson and Aaronson of NIH and their origin and purification have been described²³. Gp70s were isolated and analysed as described in Fig. 2. *f*, Composite of the major spots of the xenotropic and amphotropic virus gp70s, indicating the degree of interrelatedness between the molecules. Crosshatched areas, common to all; ○, common to amphotropic gp70 and to *a*, *b* and *d* (as well as C₃H and NZW, not shown); ●, common to NIH and NZB; ○, common to NZB (and NZB × NZW F₁, not shown).

Distinct proviruses are expressed at different sites of differentiation

To study whether different proviruses are expressed at different sites of differentiation we compared the structure of gp70s isolated from different anatomical sites in the same mouse. Figure 5 shows the tryptic peptides of the gp70 isolated from the serum and seminal fluid of 129 mice. Clearly the two proteins are distinct. The serum protein resembles gp70 of the NZB xenotropic virus whereas the seminal fluid protein is like the FMR group in

structure. Thus, serologically related but structurally distinct gp70s are found at different anatomical sites.

Formulation for members of the multi-gene family

Table 1 summarises all the data presented above. All the virion, serum and tissue proteins on the diagram are immunologically related and thus can be considered expressions of a multigene family²⁶. Groups are represented as joined boxes which are spatially placed to indicate our estimation of the extent of interrelatedness between the groups. Based on our preliminary

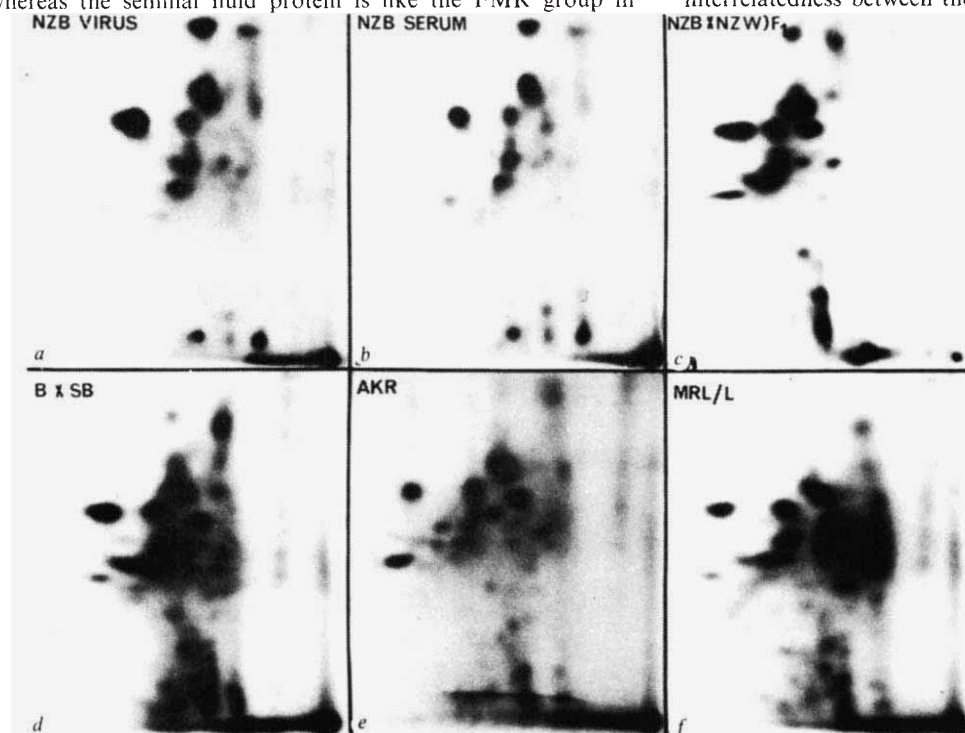
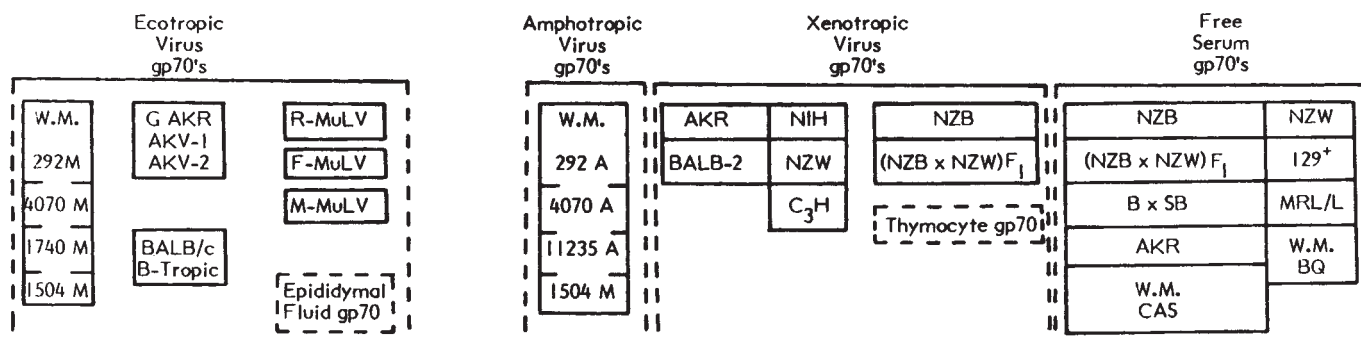


Fig. 4 Comparison of the tryptic peptides of NZB virus gp70 with the tryptic peptides of gp70s isolated from the serum of several strains of mice. *a*, NZB virus; *b*, NZB serum; *c*, (NZB × NZW)F₁; *d*, B × SB; *e*, AKR; *f*, MRL/L. Serum gp70s were isolated by immunoaffinity chromatography and treated for peptide map analysis essentially as described in Fig. 2. EDTA (10 mM) and EGTA (10 mM) were added to the sera prior to passage over the immunoaffinity column to prevent complement binding and reduce proteolysis. B × SB is a new recombinant inbred strain derived from a single (C57 B1/6 × SB/Le)F₁ cross with selection for the mutant satin (*sa*) and beige (*ba*) genes developed by Dr Edwin D. Murphy of Jackson Laboratories. MRL/L is a new recombinant inbred strain obtained from the twelfth generation of an inbred stock derived from strains LG, AKR, C₃H and C57 B1/6, also developed by Dr Murphy. Analysis of the gp70 from the sera of wild mice from two separate populations (Lake Casitas and Bouquet Canyon²⁴) had similar homology to the NZB xenotropic virus gp70 (not shown).

Table 1 Summary diagram of the structural relationship between all virion- and non-virion-associated gp70s analysed

Joined boxes represent closely related groups and the spatial arrangement of these groups is intended to indicate intergroup relatedness. The gp70s found free in the epididymal fluid and on the surface of thymocytes are surrounded by broken lines and are placed according to their structural relatedness to virion-associated gp70s. W.M., wild mouse; C.A.S., Casitas trapping area; B.Q., Bouquet Canyon trapping area.

structural studies, the thymocyte-associated gp70 is placed in the xenotropic group. We are now doing the N-terminal sequence of a number of these gp70s to determine precisely the inter- and intra-group divergence.

Discussion

We show here that the various virion-associated and free serum gp70 molecules can be divided into groups. The extensive intergroup divergence of the FMR ecotropic murine viruses may be due to their unique origins but may also reflect recombinations during their laboratory history. By contrast, minimal divergence is seen among the members of groups of endogenous viruses very recently isolated from inbred or wild mice. Thus, given the opportunity for recombination and/or replication outside the host, oncornaviruses may diverge extensively. But, under mendelian restrictions members of each group remain relatively conserved, suggesting that selection operates at the level of the integrated provirus.

It is clear that the endogenous free or virion-associated gp70s are part of a multigene family of immunologically and structurally related proteins. An important question is: how many members does the family contain? We believe that the family may be extensive, but at present we can only give a lower limit for expression of at least five members within a given mouse. For example, in an AKR mouse there are two AKV genes, one AKR xenotropic virus gene, one gene for the serum gp70, and a gene for one of the proteins secreted into the male genital tract²⁵. This accounting does not include at least five additional gp70s expressed at other sites of differentiation¹⁰, as we have not yet

isolated these to determine if they differ from the known gp70s.

In the case of the endogenous AKR provirus, it has already been shown that two presumably identical genes (AKV-1 and AKV-2) have different genetic loci²⁷. But, in spite of the different loci, our recent studies with Hartley and Rowe have shown that the gp70s of viruses derived from AKV-1 and AKV-2 are identical. Thus with respect to the xenotropic proviruses, the question is whether they are also non-alleles or simply very polymorphic products of a single locus. Our results show that there are at least two loci for xenotropic proviruses in that in most mice a virus can be recovered from many tissues which has a gp70 distinct from that present in the serum of the same mouse. The provirus encoding the serum protein can be considered xenotropic in that in the exceptional strain (that is, NZB) when this gp70 becomes part of a virion, it confers xenotropic host range. In so far as the genes for the complete xenotropic viruses of different strains are concerned, our data do not allow us to distinguish between non-alleles against extensive polymorphism at a locus.

If we accept the assumption that the various endogenous proviruses are non-alleles, then a very interesting question concerning regulation of their expression emerges. Studies in our laboratory and Boyse's have already shown that in the mouse, gp70 is expressed only in cells following certain pathways of differentiation^{4,9}. Now the issue becomes whether at each site of differentiation the gp70 is the product of a different provirus. Although we cannot yet be precise about the answer, there are already some observations which suggest that at each site of differentiation one or more different proviruses are expressed. For example, as mentioned above, within a strain the serum and one of the genital tract gp70s have different tryptic peptides. In terms of neoplasia if the proviruses expressed at different sites of differentiation are not genotypically equivalent then one can begin to understand how expression of the viral genome at one site can have oncogenic consequences distinct from another. Regardless of to what extent we are dealing with polymorphism against multiple genes, it is already clear that the study of endogenously expressed gp70 can give important insights into the extent and perhaps mechanisms of divergence of a well-defined murine glycoprotein. The uniqueness of the situation, of course, stems from the fact that because of the extrachromosomal potential of the provirus, it is often possible to have both the gene and gene product in hand.

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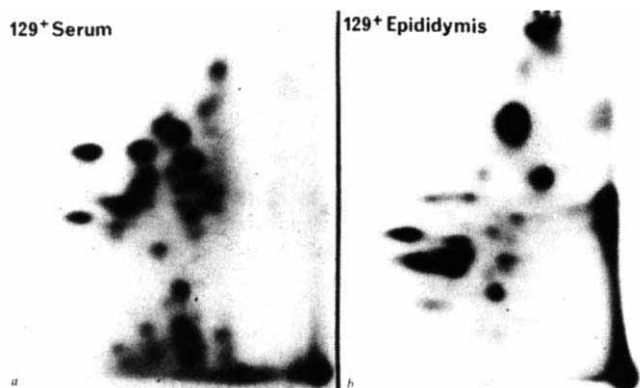
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Fig. 5 Comparison of the tyrosine-containing tryptic peptides of gp70s found free in the serum (a) and epididymal fluid (b) of mice. Gp70 was isolated from the serum of 129 G₁X⁺ mice as in Fig. 5 and analysed as in Fig. 2. The epididymal fluid gp70 was radioiodinated and immune precipitated from the epididymal secretion of male 129 G₁X⁺ mice as previously described²⁵.



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letters to nature

Galactic distribution of X-ray burst sources

By applying the following working definition for X-ray bursts¹—rise times of less than a few seconds, durations of seconds to minutes, and recurrence in some 'regular' pattern—18 burst sources have been observed to date (refs 2–16) of which one (observed by SAS-3) has not yet been reported. Their positions are shown in Fig. 1. If single 'burst' events which meet the criteria of rise time and duration but not recurrence are accepted, seven burst sources can be added^{16–19}, of which two (observed by OSO-8) have not yet been reported. These seven are also indicated in Fig. 1.

Many other 'burst' events have been reported in the literature^{20–23} but are omitted here, since they meet neither the criteria of rise time nor recurrence. It is very likely, however, that some of the many events observed by the Los Alamos group^{20–22} were X-ray bursts of the kind discussed here. Evans (private communication) believes that perhaps five or six burst sources have been found by them. Since sufficient knowledge about the rise times and the recurrence pattern of these events is lacking, they are not included in Fig. 1.

The burst sources are spread along the galactic equator and cluster near low galactic longitudes ($|l| < 40^\circ$). Lewin¹⁸ concluded, on the basis of the positions for 15 X-ray burst sources, that their distribution is different from that of the observed globular clusters²⁴. On the basis of an even smaller sample of some of the SAS-3 results, Lewin¹ also concluded that the

distribution of burst sources is unlike that of the previously known (catalogued) galactic X-ray sources²⁵, but pointed out that the burst sources could well be a subclass of the galactic X-ray sources.

The SAS-3 X-ray observatory and the Goddard X-ray Spectroscopy Experiment on OSO-8 have discovered 21 of the 25 burst sources shown in Fig. 1. Here we examine the distribution of burst sources and evaluate (with improved statistics) the effect of uneven sky exposure on the observed distribution, using data from these two observatories. In our analysis we include sources from which single 'burst' events have been observed; none of our conclusions would change if we ignored them.

From the SAS-3 observatory, we present only data from the rotating modulation collimator detectors²⁶ (RMC), which have $\sim 12^\circ \times 12^\circ$ (FWHM) fields of view. The RMC systems view the sky in the direction of the spin axis ($+z$ axis). Their view direction therefore does not change rapidly, and the sky exposure can be easily calculated. We have thus ignored several sources from which bursts were detected in the SAS-3 y -axis detector systems only^{7,8,15}.

The OSO-8 burst observations were made with three independent detectors viewing the directions of the $+z$ axis (spin axis) and that of the $-z$ axis. The detector that views the sky in the $+z$ direction has a circular field of view of $\sim 5^\circ$ FWHM. Two detectors view the sky in the direction of the $-z$ axis; one has a circular field of view of $\sim 3^\circ$ FWHM (centred on the $-z$ axis) and one has a circular field of view of $\sim 5^\circ$ FWHM (this one is offset from the $-z$ axis by 5°). These OSO-8 detectors have been described in more detail by Serlemitsos *et al.*²⁷. For the present purpose, the two $-z$ axis detectors can be considered as one detector system with a circular field of view of radius $\sim 10^\circ$ centred on the $-z$ axis. This is certainly valid for observations of bursts that last longer than ~ 20 s, since the spin period of OSO-8 is ~ 10 s. For shorter bursts, the equivalent field of view decreases by a factor of up to 2.

The sensitivity of each detector for detecting bursts depends on the detector area, the background level, the field of view and the exposure time. For the SAS-3 RMC and the OSO-8 $5^\circ -z$ axis detectors, the sensitivity is also adversely affected by modulations of strong sources due to rotation of the satellites. We do not attempt here to draw any conclusions from a comparison of the number of burst sources seen by the three different detectors.

The calculated sky exposure (in days) and the number of burst sources observed are shown in Tables 1 and 2. The results were calculated using 'clean' data only. As a result of Earth occultation, the actual values for the sky exposure were $\sim 30\%$ less (mean value) than those shown in the tables. The SAS-3 results are primarily from 'quick look' data ($\sim 50\%$ of the total data collected) during the first 19 months of observations (up to January, 1977). The OSO-8 results are from the first 16 months of observations (up to October 1976); for the last 4

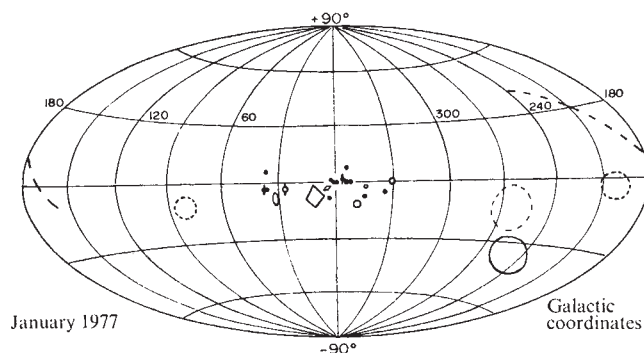


Fig. 1 Sky map (galactic coordinates) of 18 X-ray burst sources which meet the three burst criteria (see text). Seven additional sources are shown for which the recurrence criterion of the bursts has not (yet?) been met. They are indicated with dashed lines (four sources) or with vertical bars (three sources). Open error boxes have areas ≥ 3 deg². The dashed line near the galactic anticentre represents an error box of $\sim 120^\circ \times 2^\circ$. The figure contains only burst sources discovered before 6 January, 1977. For more detailed information see ref. 18.

Table 1 Galactic latitude distribution of observed X-ray burst sources

$ b^{\text{II}} $	SAS-3 RMC data only		OSO-8 data		Arp catalogue Globular clusters
	Exposure* (d)	Burst sources observed	Exposure* (d)	Burst sources observed	
$< 20^\circ$	108	9	71	13	~ 75
$> 20^\circ$	207	1	34	0	~ 45

*Not corrected for Earth occultation

months, only 'quick look' data ($\sim 10\%$ of the total) are available. For OSO-8, the sky exposure at any absolute value of the galactic latitude is obviously the same for the $+z$ and the $-z$ detectors. We have therefore combined the data of the OSO-8 $+z$ and $-z$ detectors in Table 1 (but not in Table 2).

The numbers of burst sources listed in Table 1 observed by SAS-3 (RMC) and OSO-8 at low galactic latitudes ($|b^{\text{II}}| < 20^\circ$) are nine and thirteen respectively. (These numbers are lower limits because of possible source confusion. We estimate that perhaps two or three more sources were detected in the region of high burst source density within $\sim 10^\circ$ of the galactic centre.) Three of the thirteen sources observed by OSO-8 were also observed by SAS-3 (RMC). Therefore, nineteen different burst sources are observed in the region $|b^{\text{II}}| < 20^\circ$. One burst source was observed at galactic latitude $|b^{\text{II}}| > 20^\circ$; this source, observed by SAS-3, has not been reported previously. The sky exposure for SAS-3 in the region $|b^{\text{II}}| > 20^\circ$ was about twice that in the region $|b^{\text{II}}| < 20^\circ$; for OSO-8 the exposure at low galactic latitude was about twice that at high galactic latitude.

The combined results of SAS-3 and OSO-8 illustrate unambiguously that the galactic latitude distribution of X-ray burst sources is very different from that of the known globular clusters²⁴ (see last column Table 1).

The galactic longitude distribution of X-ray sources for which $|b^{\text{II}}| < 20^\circ$ is shown in Table 2. Again, the numbers of observed sources given for the region $|l^{\text{II}}| < 60^\circ$ are lower limits (see above). In regions of low galactic longitude ($|l^{\text{II}}| < 60^\circ$) the OSO-8 $-z$ and $+z$ detectors observed nine and six

We conclude from the combined OSO-8 and SAS-3 results of Table 2 that the galactic longitude distribution of X-ray burst sources is different from that of the low galactic latitude X-ray sources listed in the 3U catalogue²⁵ (see Table 2). It is quite possible, however, that the burst sources are a subclass of the low galactic latitude X-ray sources. Five of the twenty-five burst sources shown in Fig. 1 and possibly many more of them (refs 1, 9, 16, 18, 19) are associated with sources of steady X-ray emission.

It is interesting to note that the galactic longitude distribution for known globular clusters (in the region $|b^{\text{II}}| < 20^\circ$) is similar to that of burst sources (see last column, Table 2). It has been proposed (see discussion in ref. 1) that perhaps the majority of burst sources are located only in those globular clusters near the galactic plane, but as pointed out earlier¹ this is very unlikely. There are at present sixteen X-ray burst sources whose positional uncertainties exclude known globular clusters; two burst sources are located in globular clusters (one in NGC6624 and the rapid burster), and seven additional burst sources could be located in globular clusters (it is very unlikely that all seven are). Therefore, of the ~ 65 known globular clusters (ref. 24) in the region $|b^{\text{II}}| < 15^\circ$, two or at most eight are known to produce bursts. Thus, for every known globular cluster that is known to produce bursts there are ~ 8 – 30 that are not known to produce bursts. If the 16 burst sources whose positions exclude known globular clusters were located in obscured globular clusters, and if the occurrence of a burst source is as probable in obscured clusters as in the known ones, then there would have to be an additional 130–480 obscured globular clusters within 15° of the galactic plane. Their distribution would be very different from the spherical distribution of the known globular clusters, and they would constitute, in effect, a distinct disk population of globular clusters. To avoid this, one might assume that an intense concentration of matter in the immediate vicinity of many globular clusters is responsible for the obscuration of these clusters and that the presence of this obscuring matter somehow increases the probability of the occurrence of burst sources. We believe it is more likely that the majority of X-ray burst sources are not

Table 2 Galactic longitude distribution of observed X-ray burst sources for $|b^{\text{II}}| < 20^\circ$

$ l^{\text{II}} $	SAS-3 (RMC)		OSO-8 ($-z$)		OSO-8 ($+z$)		3U Catalogue	Arp catalogue globular clusters
	Exposure* (d)	Burst sources observed	Exposure* (d)	Burst sources observed	Exposure* (d)	Burst sources observed		
0° – 60°	43	8	14	9	25	6	53	~ 71
60° – 120°	32	1†	32	1	32	0	29	5
120° – 180°	32	0	25	1	14	0	15	2

*Not corrected for Earth occultation.

†The source at $l^{\text{II}} \sim 250^\circ$ (see Fig. 1) is located at $|b^{\text{II}}| > 20^\circ$ and is therefore not included.

burst sources, respectively. Four of these sources were observed by both detectors. Therefore, eleven different burst sources were detected by OSO-8, of which three were also observed by SAS-3. Thus, a total of sixteen different burst sources were observed by the two observatories in the region of low galactic longitude ($|l^{\text{II}}| < 60^\circ$). One burst source was observed by SAS-3 near galactic longitude $l^{\text{II}} \sim 114^\circ$ (refs 17, 18) and one was observed by OSO-8 (unpublished) near $l^{\text{II}} \sim 79^\circ$. OSO-8 observed one burst source (unpublished) at high galactic longitude $|l^{\text{II}}| = 120^\circ$ – 180° . (One other burst source was observed at high galactic longitude with one of the SAS-3 y -axis detector systems (dashed line in Fig. 1, refs 1, 18). The Los Alamos group may have detected three additional burst sources (refs 20–22) in this region. However, as mentioned earlier, the 'burst' character of these events is not yet established. The source at $l^{\text{II}} \sim 250^\circ$ (see Fig. 1) is located at $|b^{\text{II}}| > 20^\circ$ and is therefore not included in Table 2.)

located in globular clusters of the kind that we know. This conclusion is supported by the direct observational evidence of Liller, who systematically searched for globular clusters in the vicinity of six burst sources and found only one²⁸. In five cases no globular clusters were apparent on his infrared plates (ref. 29). Liller believes that, in the case of MXB1728-34 for example, he would have detected a globular cluster out to a distance of ~ 19 kpc (ref. 29).

It has also been suggested (discussion in ref. 1; ref. 30 and T. Gold, unpublished) that the bursts are perhaps produced by remnants of disrupted globular clusters, specifically super-massive black holes. Such objects could perhaps accrete a sufficient amount of gas in the galactic plane (but not away from the plane) to produce bursts. Again, this would imply the existence of a new class of unknown objects and is at present merely an *ad hoc* method of relating the burst sources to globular clusters.

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W. H. G. LEWIN

J. A. HOFFMAN

J. DOTY

G. W. CLARK

Department of Physics and Center for Space Research,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

J. H. SWANK

R. H. BECKER

S. H. PRAVDO

P. J. SERLEMITOS

Goddard Space Flight Center,
Greenbelt, Maryland 20771

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First mass spectrometric measurements of positive ions in the stratosphere

ROCKET and balloon-borne electrostatic probe measurements have shown that positive and negative ions are present in the stratosphere reaching number densities between 100 and 10,000 cm^{-3} (refs 1-5). The observed densities indicate that galactic cosmic radiation is the major ionisation source and mutual recombination is the dominant ion loss process. No experimental information exists, however, on the nature of stratospheric ions. Here we communicate the first mass spectrometric measurements of positive ions in the stratosphere.

Three experiments were carried out at high latitudes (experiment F 33, 31 May 1972, 06.53 UT, latitude 69°N; experiment F 42, 10 June 1975, 13.19 UT, latitude 69°N; experiment S 18-1, 21 February 1976, 19.42 UT, latitude 68°N) using rocket-borne cryogenically pumped quadrupole mass filters. Mass spectra of positive ions at stratospheric heights were obtained between 24 km and 60 km during the descent portions of the rocket flights.

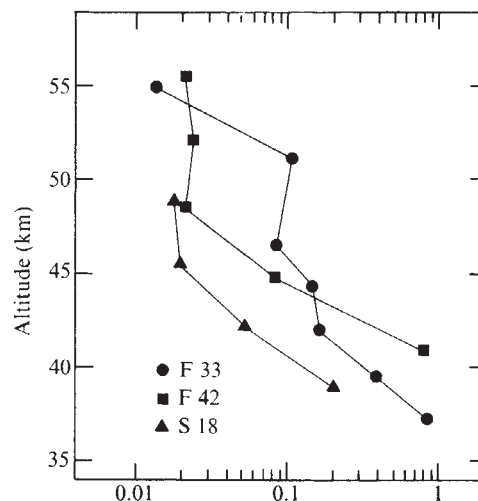
The mass numbers of the most abundant ions detected are listed in Table 1 along with tentative identifications. The uncertainty of ± 2 in atomic mass numbers is due to a relatively low mass resolution used in favour of high sensitivity. Above about 40 km the masses 19 ± 2 , 37 ± 2 , 55 ± 2 and 73 ± 2 are most abundant which are identified as proton hydrates (PH)

Table 1 Observed mass numbers and tentative identifications of ions

Mass (AMU)	Tentative identification of ions
19 ± 2	H_3O^+
29 ± 2	$\text{H}^+\text{CH}_2\text{O}$
37 ± 2	$\text{H}^+(\text{H}_2\text{O})_2$
42 ± 2	$\text{C}_2\text{H}_2\text{O}^+$
55 ± 2	$\text{H}^+(\text{H}_2\text{O})_3$
60 ± 2	$\text{H}^+(\text{CH}_2\text{O})_2$; $\text{C}_2\text{H}_2\text{O}^+\text{H}_2\text{O}$
80 ± 2	$\text{H}^+(\text{CH}_2\text{O})_3\text{H}_2\text{O}$; $\text{C}_2\text{H}_2\text{O}^+(\text{H}_2\text{O})_2$

$\text{H}^+(\text{H}_2\text{O})_n$ with n ranging from 1 to 4. It should be noted that PH were also found above 55 km in the mesosphere by these experiments. Here the mass filters were additionally operated in a second mode using higher mass resolution. Therefore, the identification of the above masses as PH seems to be quite safe. Below about 40 km the PH rapidly disappear and ions having masses 29 ± 2 , 42 ± 2 , 60 ± 2 and 80 ± 2 become most abundant. The change in the ion population occurs within only a few kilometers as demonstrated by Fig. 1 showing the fractional abundance of non-proton hydrates (NPH). The NPH probably result from PH reacting with one or more stratospheric trace gases by switching or proton transfer reactions. The unknown reaction partner should have either a higher permanent dipole moment or a higher proton affinity than H_2O or both and its number density should steeply decrease with height around 40 km. An inspection of molecules which satisfy condition one and which after adding a proton fall into the mass interval 29 ± 2 AMU corresponding to the observed ion of smallest mass yields the following candidates: CH_2O , HCN , CH_5N and CH_3NH_2 . Certainly, these species do not represent a complete list but were chosen also on the base of aeronomic arguments. Although, however, none of these species has been measured in the stratosphere so far, at least CH_2O is expected to be present in substantial concentrations resulting from the oxidation of methane⁶. The proton affinity of CH_2O ($165 \pm \text{kcal mol}^{-1}$) only slightly exceeds that of H_2O ($164 \pm \text{kcal mol}^{-1}$) as also proven by the qualitative observation of H_3O^+ proton transfer with CH_2O (ref. 7). The higher masses observed in our experiments also seem to fit into the concept of a stratospheric formaldehyde ion chemistry. Mass 42 ± 2 might be identified as $\text{C}_2\text{H}_2\text{O}^+$ which has already been observed⁸ in the laboratory as a product of $\text{H}^+\text{CH}_2\text{O}$ reacting with CH_2O . The masses 60 ± 2 and 80 ± 2 might be identified as the cluster ions $\text{H}^+(\text{CH}_2\text{O})_2$ or $\text{C}_2\text{H}_2\text{O}^+\text{H}_2\text{O}$ and $\text{H}^+(\text{CH}_2\text{O})_3$, H_2O or $\text{C}_2\text{H}_2\text{O}^+(\text{H}_2\text{O})_2$ respectively. The number density of the reaction partner X of PH can be estimated from the measured density ratio of NPH and PH. Assuming a chemical

Fig. 1 Fractional abundance of non-proton hydrates. ●, F33; ■, F42; ▲, S18.



equilibrium, the continuity equation

$$[\text{PH}^+] K [\text{X}] = [\text{NPH}^+] \alpha [\text{N}^-]$$

relates $[\text{X}]$ to the measured density ratio $[\text{NPH}^+]/[\text{PH}^+]$ the reaction coefficient K , the mutual recombination coefficient α and the negative ion density $[\text{N}^-]$. Taking a typical $\alpha = 5 \times 10^{-8} \text{ cm}^{-3} \text{ s}^{-1}$ based upon laboratory measurements⁹, a lower limit for $K = 10^{-10} \text{ cm}^{-3} \text{ s}^{-1}$ and a typical measured $[\text{N}^-]^2$ (which due to the absence of free electrons equals $[\text{N}^-]$) the $[\text{X}]$ profiles shown in Fig. 2 are obtained. When compared with the relative profile of the total gas density $[\text{M}]$ the $[\text{X}]$ profile steeply decreases above about 40 km as does a model profile of CH_2O (ref. 6). Even the absolute $[\text{X}]$ values, although uncertain mainly due to the assumed K and $[\text{N}^-]$ values, seem to be of the right order of magnitude. This similarity may provide further support for the identification of NPH as products of CH_2O reactions. The following simplified reaction scheme is proposed

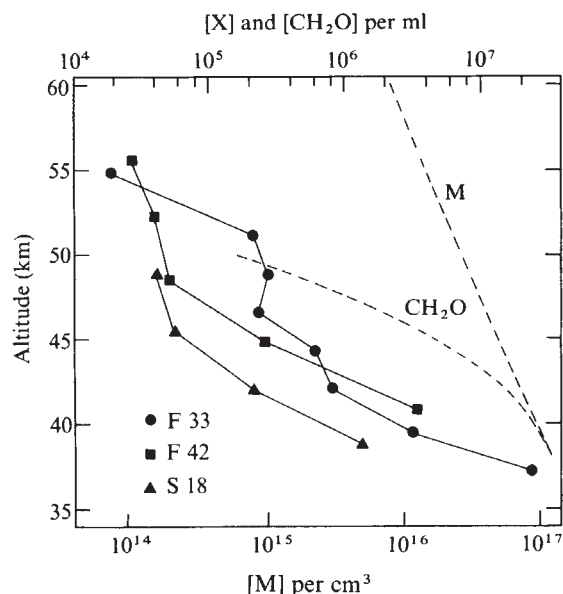


Fig. 2 Number densities of the unknown reaction partner X as derived from the data. The total gas density M (ref. 12) and a theoretical CH_2O profile⁶ are shown for comparison. ●, F33; ■, F42; ▲, S18.

(Fig. 3). The primary ions N_2^+ , O_2^+ , N^+ and O^+ which are formed by galactic cosmic radiation rapidly convert to proton hydrates. While the ion lifetime against mutual recombination is about 2,000 s or greater the PH -formation and PH -growth to an equilibrium size distribution proceeds within less than 0.01 s at 40 km. As soon as the number density of a potential reaction partner X for the PH exceeds a critical value of about $5 \times 10^6 \text{ cm}^{-3}$ (value for which $[\text{NPH}] = [\text{PH}]$) the PH -conversion becomes effective. According to measured equilibrium constants for PH (ref. 10) the most abundant PH at 40 km should be $\text{H}^+(\text{H}_2\text{O})_4$ and $\text{H}^+(\text{H}_2\text{O})_5$.

As formaldehyde not only has a higher proton affinity but also has a higher permanent dipole moment¹¹ (2.27 debye) than water (1.84 debye) it should replace H_2O when reacting with $\text{H}^+(\text{H}_2\text{O})_n$. As the difference of the CH_2O - and H_2O bond energies is probably smaller than the bond energy of the $(n-1)$ th H_2O no additional H_2O will be 'boiled off'. The product ion $\text{H}^+(\text{H}_2\text{O})_{n-1}\text{CH}_2\text{O}$ may undergo additional exchange reactions with CH_2O which may finally lead to $\text{H}^+(\text{CH}_2\text{O})_n$. Also further association of CH_2O may occur resulting in complexes $\text{H}^+(\text{CH}_2\text{O})_k$ of higher order of association ($k > n$) than the original PH . It may also be considered that the excited complex $[\text{H}^+(\text{H}_2\text{O})_n\text{CH}_2\text{O}]^*$ formed by association of CH_2O to $\text{H}^+(\text{H}_2\text{O})_n$ does not stabilise by ejection of an H_2O molecule but by ejection of molecule fragments. Cluster ions containing $\text{C}_2\text{H}_2\text{O}^+$ as a central ion might have been formed by such

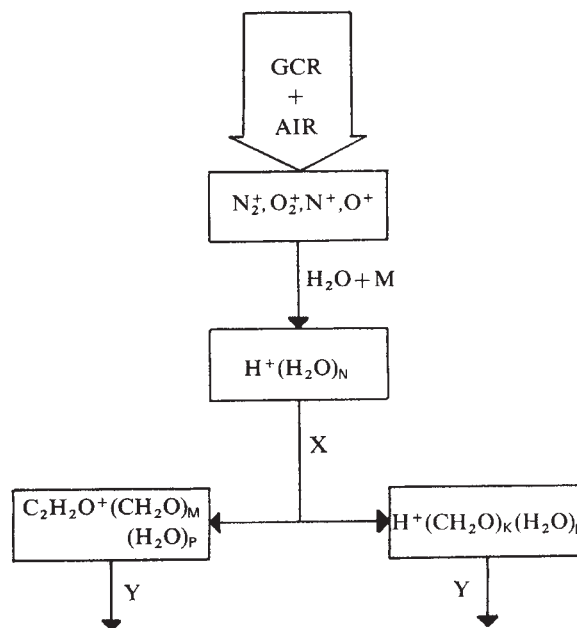


Fig. 3 Simplified reaction scheme for stratospheric positive ions at heights above 24 km. Reaction partners are indicated beside or above the arrows. M denotes an N_2 or O_2 molecule and Y denotes a molecule having a higher permanent dipole moment or proton affinity or both than CH_2O . GCR denotes galactic cosmic radiation.

processes. This was suspected previously from laboratory experiments⁸. Drift tube mass spectrometer studies carried out in our laboratory¹³ indicate that proton hydrates $\text{H}^+(\text{H}_2\text{O})_n$ with n ranging from 1 to 4 rapidly react with formaldehyde. It was also found that the product ions of the $\text{H}^+(\text{H}_2\text{O})_n + \text{CH}_2\text{O}$ process undergo a fast reaction with NH_3 resulting in $\text{NH}_4^+(\text{NH}_3)_n$ ions. Therefore an upper limit of atmospheric NH_3 at 24 km may be estimated from our stratospheric ion data. In contrast to the above estimate of CH_2O , three-body recombination has also to be considered at 24 km yielding an increased total recombination coefficient and thus an increased critical number density for a potential reaction partner of formaldehyde related ions. Taking a three-body recombination coefficient of $2.10^{-25} \text{ cm}^6 \text{ s}^{-1}$ (ref. 14) one obtains an effective recombination coefficient of $2.1.10^{-7} \text{ cm}^3 \text{ s}^{-1}$ at 24 km. As no NH_3 related ions were found at 24 km the relative abundance of $\text{NH}_4^+(\text{NH}_3)_n$ ions was less than 0.07 corresponding to $[\text{NH}_3] \leq 7.10^4 \text{ cm}^{-3}$.

Finally, two points should be stressed. First, that the observed ions could possibly represent fragments of ambient ions formed by electric field or shock wave-induced collisional break-up during sampling. As fragmentation causes successive stripping-off of neutral molecules (presumably CH_2O and H_2O) attached to the central ion, the nature of the central ion remains unchanged and so do the general conclusions drawn here. Second, one must consider the powerful diagnostic possibilities of stratospheric ion composition measurements for the detection of stratospheric neutral trace gases and even for a determination of their concentrations. Future *in situ*-measurements using higher mass resolution as well as laboratory studies of the relevant ion chemical processes are needed before these possibilities can be exploited.

F. ARNOLD
D. KRANKOWSKY
K. H. MARIEN

Max-Planck-Institut für Kernphysik,
Heidelberg, FRG

Received 20 December 1976; accepted 4 March 1977.

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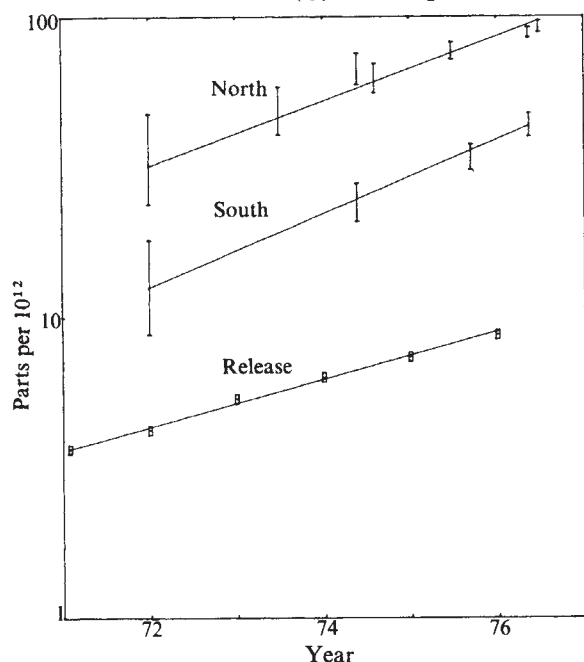
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Methyl chloroform in the troposphere as an indicator of OH radical abundance

METHYL chloroform (111 trichloro ethane) was introduced into general use in 1955-60 in response to the need for a general solvent which was less toxic than those then in use. Its ubiquitous presence in the northern and southern hemispheres, first reported in 1974 (ref. 1), has since been confirmed^{2,3}. The only significant sources of methyl chloroform are industrial releases from its use as a cleaning agent and solvent. A principal sink for its removal from the atmosphere seems to be the reaction with OH radicals. The rate of its release to the atmosphere is known with some accuracy, and consequently the measurements of its growth in the atmosphere could provide valuable information on the strength of the OH reaction sink, not only for methyl chloroform but also for other important atmospheric gases which undergo similar reactions. This communication reports measurements of the abundance of methyl chloroform made during 5 yr in the northern and southern tropospheres.

Methyl chloroform was analysed directly from 5-ml air samples using a gas chromatograph equipped with an electron capture detector. The method was that described for other halocarbons, including the fluorocarbons and carbon tetrachloride⁴. Figure 1 shows the concentration observed during the past 5 yr at measuring sites in rural

Fig. 1 Observed concentrations: parts per 10^{12} by volume of CH_3CCl_3 in the northern hemisphere (British Isles) and southern hemisphere (South Africa and Antarctica) during 1972-77. Cumulative releases (○) are in megatonnes.



regions of the British Isles, in the southern hemisphere in Cape Province South Africa and aboard the RV Shackleton. Northern hemisphere measurements were all made, except in 1972, during periods of unpolluted Atlantic air.

The observations in the southern hemisphere have been followed from just about the noise level in 1971, at an abundance of 1.2×10^{-11} , to the present level of 5×10^{-11} . The low initial finding suggests that there is no significant natural source of this compound. The absolute accuracy of the analytical method is not likely to be worse than 30% which is in general agreement with the experience of other analysts^{2,4}. Figure 1 also shows the annual releases of methyl chloroform to the atmosphere from industrial sources (personal communication from W. B. Neeley and J. Plonka). When applied to the observations here reported, using a simple two box model of the atmosphere, these data suggest an atmospheric residence time of between 5 and 10 yr. H. B. Singh (personal communication) reports observations also pointing to a longer residence time for methyl chloroform. Previous estimates of the residence time have varied from 1 to 2 yr (refs 3 and 5). It is unlikely that the measured rate of reaction of OH radicals with methyl chloroform⁶ is seriously in error, and the most likely source of the anomaly is in the time averaged value assumed for the OH concentration. The yearly average concentration is usually taken to be in the range $3-5 \times 10^5$ radicals per ml. This is a mean of several estimates⁷⁻⁹. One outstanding exception, however, is the estimate by Warneck¹⁰ of 7×10^5 per ml, for the yearly average. This lower value is more consistent with the observations reported here. The discrepancy in OH concentration is considerable and if it propagates to the reaction of the other atmospheric gases such as methane, CO and other hydrocarbons, could influence the interpretation of the chemistry of both the troposphere and the stratosphere. The comparatively uncomplicated industrial source and natural OH sink of this gas suggests that it may serve as a useful indication of the distribution of OH radicals.

Crutzen⁸ has discussed the consequences of the presence of this compound on the ozone layer. If current theories of ozone depletion by chlorine-containing species are confirmed by atmospheric measurements and if methyl chloroform is produced in ever increasing quantities for several decades, I agree with Crutzen that the atmospheric burden would give cause for concern.

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J. E. LOVELOCK

Bowerchalke,
Salisbury,
Wiltshire, UK

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Inhomogeneities in the water properties of fogs and clouds

ALTHOUGH there is good evidence that entrainment of undersaturated environmental air exercises a profound influence upon the development of the droplet spectra in clouds, the classical description of the mixing process¹ has

Table 1 Values of droplet concentrations N , water content C , mean radius r , and dispersion S measured within hill fog (mean spacing of consecutive spectra about 3 m)

N	129	140	112	136	115	105	99	106	111	99	97	76	112	95	106	154	144	118	142	168
C	11	12	9.0	11	8.9	7.6	7.0	8.8	8.1	8.4	7.8	6.2	8.2	7.3	8.3	13	12	10	11	16
r	5.5	5.4	5.4	5.4	5.4	5.1	5.3	5.5	5.4	5.5	5.5	5.5	5.3	5.3	5.3	5.4	5.4	5.6	5.3	5.6
100S	22	29	22	26	22	32	19	24	18	25	22	20	25	23	29	25	24	21	29	29

failed to yield quantitative predictions of spectral evolution which are consistent with observation. In the classical model the mixing process is homogeneous, that is, all droplets at a given level within the cloud are, at any time, exposed to identical conditions of supersaturation or undersaturation. Recent laboratory experiments², however, have indicated that the mixing process in clouds may be highly inhomogeneous, with some droplets completely evaporated, or substantially reduced in size, and others negligibly influenced. This work² led to the formulation of a primitive model of inhomogeneous mixing in which it was predicted that sizeable fluctuations in the water properties of natural clouds should exist, on scales commensurate with those of the mixing process. In particular, it was proposed that there may exist closely adjacent regions in which the liquid water content was very different but the spectral shape essentially unchanged. Some recent field evidence supports this idea³ but few observational data are currently available. We describe here some preliminary results indicative of the highly inhomogeneous nature of the water properties of some clouds, on certain spatial scales.

An electrostatic disdrometer⁴ has been used to measure the size distribution of droplets within cloud and fog at

the disdrometer, of droplet concentration, N , water content, C , mean drop radius r , and spectral dispersion, S (defined as the ratio of the standard deviation to the mean size), for consecutive spectra spaced about 3 m apart in orographic hill fog. Measurements were made ~150 m above the cloud base. Since collection efficiency calibrations of the device are not complete the values of each of these four parameters, although acceptable relative to each other, are not absolute, and thus no units are attached to them. It is seen that considerable variations exist, on a scale of less than 10 m, in the water content of the fog. C varies by up to a factor of about 2.5, while the mean radius r is fairly constant. The dispersion of the spectrum undergoes significant variations, but closely spaced regions of similar values of S but widely different water content are also observed.

Table 2 presents a typical set of spectral parameter values measured several hundred metres above the base of a precipitating cloud, with consecutive spectra spaced about 50 m apart. Here, the fluctuations are much smaller than for the case presented in Table 1 and the measured dispersion is substantially higher. Although the liquid water content is appreciably higher than for the hill fog the mean radius

Table 2 Values of droplet concentration, N , water content, C , mean radius, r , and dispersion, S , measured within precipitating cloud (mean spacing of consecutive spectra about 50 m)

N	241	252	251	259	243	225	210	228	234	216	250	252	250	242	242	230	237	227	235	247
C	16	18	16	18	18	17	17	18	18	19	19	17	17	17	18	15	16	15	14	18
r	4.9	5.2	4.9	5.0	5.2	5.2	5.3	5.3	5.1	5.5	5.1	5.1	5.0	5.1	5.0	5.0	5.0	5.0	4.7	5.2
100S	34	30	36	35	31	34	32	31	38	29	35	34	33	31	40	32	36	35	38	32

the UMIST field-research station on the summit of Great Dun Fell (870 m above sea level) in Cumbria. Orographic effects are generally of great importance in the formation of these clouds. Droplets are sampled and sized continuously at a rate of several hundred per second with the disdrometer, which can be mounted either on a platform close to the ground or on a 30 m mast. The level of spatial resolution of the water properties of the clouds is dependent on the horizontal wind speed, which was measured. In light winds statistically meaningful spectra can be obtained at intervals down to several metres; and in winds of around 25 m s^{-1} , characteristic of this site, the minimum spatial scale that can be studied is some tens of metres.

Table 1 presents a typical set of values, obtained with

is significantly lower (by about $0.3 \mu\text{m}$). This could be a consequence of preferential capture by raindrops of the larger droplets within the condensate spectrum.

Figure 1 presents characteristic uncorrected spectra obtained for the two situations covered by Tables 1 and 2. In both cases the principal contribution to the water content of the spectrum is provided by droplets in a narrow central size band; the contribution from the largest droplets in the spectrum is very small. Statistical analysis shows that fluctuations in water content observed cannot be a consequence of randomness in rate of arrival of droplets; this conclusion was confirmed in laboratory tests using a cloud tunnel.

Preliminary results obtained so far indicate that, on certain spatial scales at least, very considerable fluctuations exist in the water properties of clouds and fogs, and we hope that future work will provide more quantitative information on the nature of these inhomogeneities in a variety of meteorological conditions.

R. G. CORBIN
J. LATHAM
C. MILL
M. H. SMITH*
I. M. STROMBERG

Physics Department,
UMIST,
Manchester, UK

Received 30 December 1976; accepted 10 March 1977.

* Present address: Physics Dept, Preston Polytechnic.

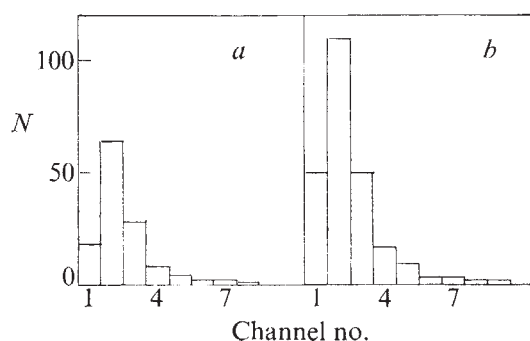
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Fig. 1 Characteristic droplet spectra measured in (a) orographic hill fog, (b) precipitating cloud. N is the droplet count. The channels are $0.9 \mu\text{m}$ wide, and channel 2 corresponds to a radius of $5.2 \mu\text{m}$.



Fine structure in caustic junctions

EVERYONE has seen the changing patterns of bright caustic lines traced on the sea bed or on the bottom of a swimming pool^{1,2}. The caustics are formed when sunlight is focused after refraction by smooth waves on the water surface; similar caustics can result from reflection. Here we draw attention to one feature of these patterns: the frequent occurrence of junctions at which three caustics appear to meet (Fig. 1).

It might seem that this is not worthy of comment—after all, the stability of triple junctions under perturbation makes them widespread in many natural systems of lines and surfaces^{2,3} such as mud cracks, foams, and the markings on giraffes. But triple junctions in caustic patterns would be surprising. The reason is that, except in special circumstances, the forms of caustics are restricted to be 'elementary catastrophes'⁴⁻⁶ and in two dimensions (i.e. two 'control variables') these are smooth curves ('fold' catas-

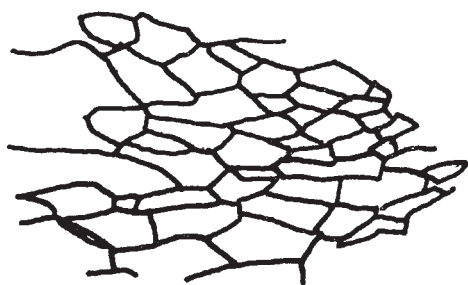


Fig. 1 Caustics formed by a wavy water surface (after Minnaert¹).

trophenes) which may have cusp points: triple junctions occur nowhere in the list of elementary catastrophes. The qualification 'except in special circumstances' implies the assumption of genericity that underlies catastrophe theory. It means in this case the absence of high symmetry in the wavefront whose normals envelop the caustic and is certainly satisfied for the random water waves considered here.

These considerations suggest that the triple junctions are illusory. In fact they are the appearance under low resolution of a caustic structure with fine detail on several levels. The following argument shows that this structure involves the meeting of six lines rather than three. Below each

Fig. 2 Line pairs where caustic surfaces from ridges intersect the sea bed.

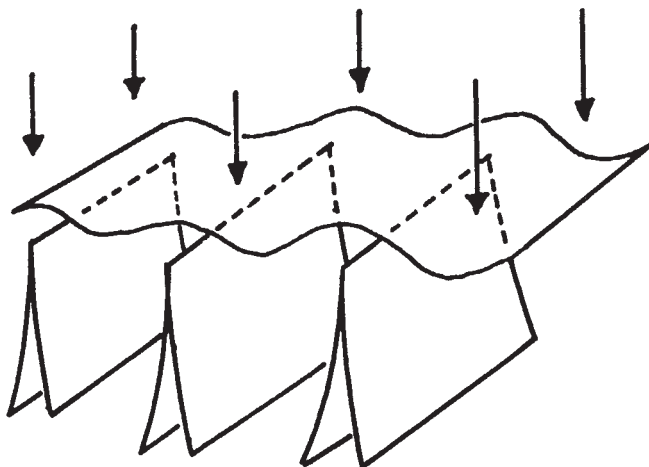


Fig. 3 Glass 'lollipop' on whose surface three grooves meet.

'ridge' on the water surface is a caustic surface with two sheets that meet along a cusped edge (Fig. 2); if the sea bed intersects this surface it will generally do so in two almost-parallel lines (these are easily seen in sunlight in, say, 2 m depth of water on which the waves are long-crested). The ridges meet in threes on the water surface, and the associated meetings of three line pairs on the sea bed are the 'triple junctions' under discussion.

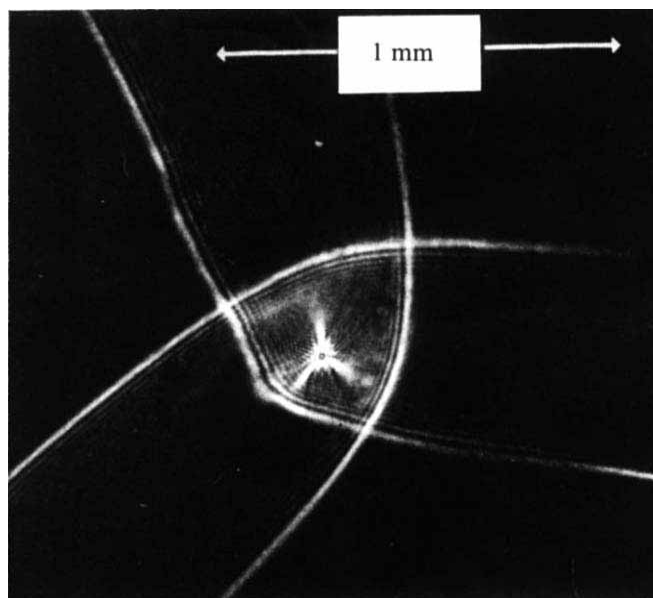
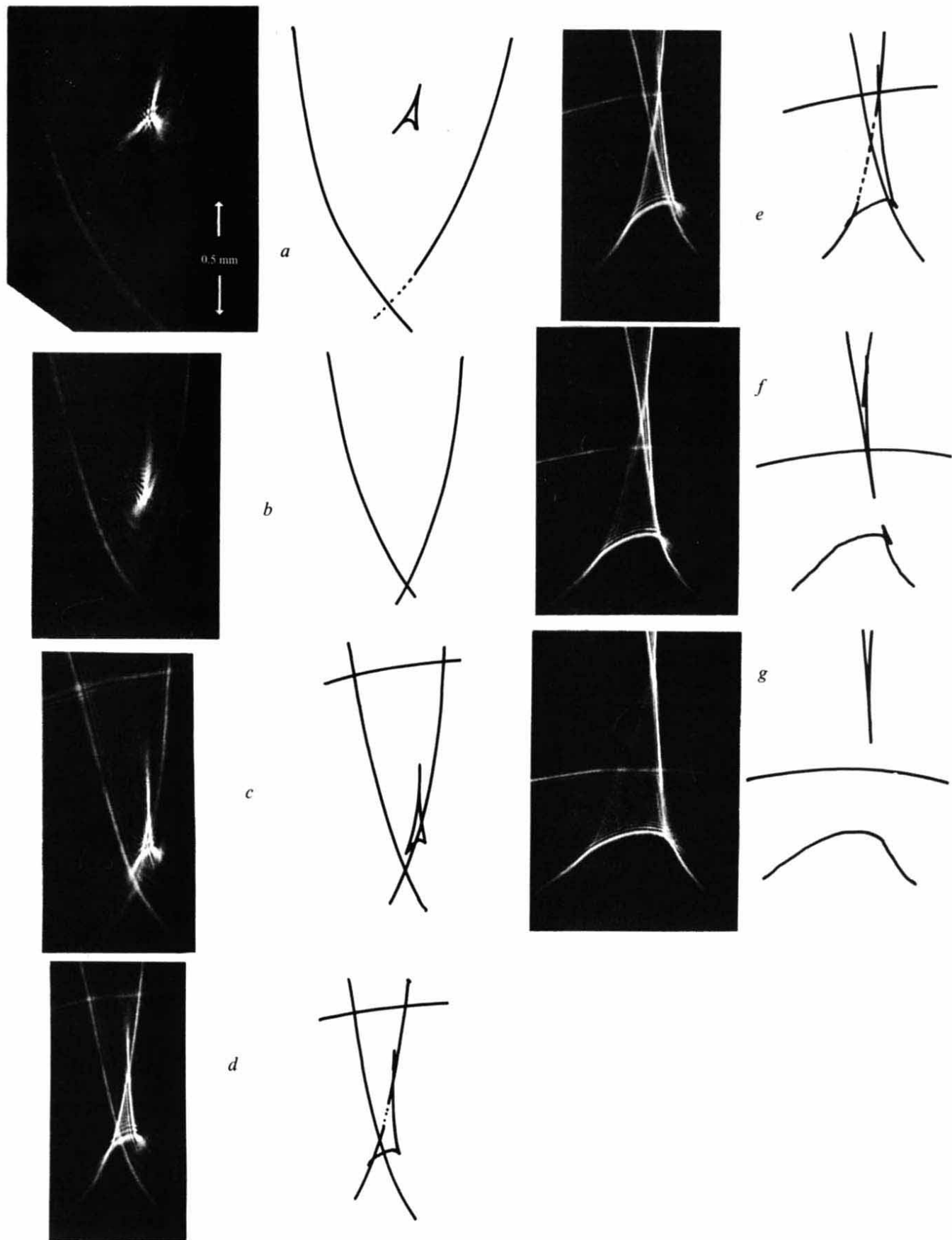


Fig. 4 Typical fine structure in caustic junction, formed by refraction of white light from a small distant source through the 'lollipop' of Fig. 3 and photographed with the aid of a microscope.

To study the details of such a junction 'in vivo' would be difficult in view of its rapid motion and the blurring caused by the 0.5° divergence of rays from different parts of the Sun. Therefore conditions near the junction of three ridges were simulated 'in vitro' on the surface of the disk of a glass 'lollipop' (Fig. 3) formed in the molten state. The diameter of the disk was 40 mm. White light from an illuminated pinhole was viewed through a microscope after being refracted by the disk. (It was easier to produce grooves on



the glass surface rather than ridges, so that the caustics were virtual rather than real.) In this way the three line pairs were easily resolved and typical patterns do indeed show the smooth curves and cusps anticipated on the basis of catastrophe theory (see Fig. 4). Note the inner cusped

'triangle', for this provides the key to the understanding of the higher-dimensional caustic structures now to be discussed.

Altering the focus of the microscope introduces an additional control variable which enables the three-dimensional

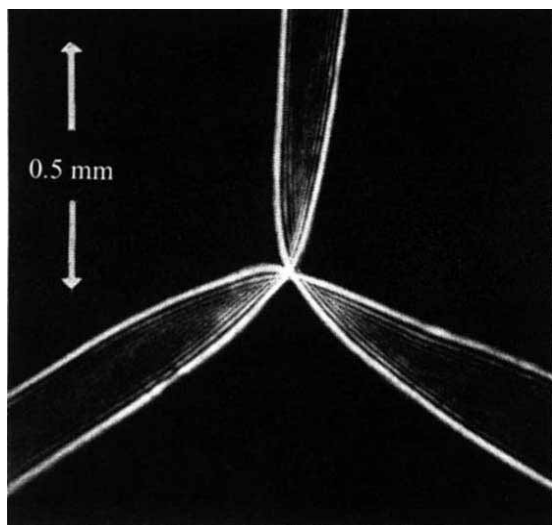


Fig. 6 Singular section of a higher catastrophe.

caustic surface to be explored in two-dimensional sections. In nature this new variable could represent time. A 'through-focus' sequence of patterns is shown on Fig. 5a-g. The complicated changes in caustic structure must be governed by catastrophe theory with three control variables. In our experiments the finest details of some of these changes were obscured by characteristic diffraction effects; the intensity and linear scale of the diffraction effects increase, for fixed wavelength, with the order of the singularity. According to catastrophe theory, cusp points may appear and disappear in pairs, either at beak-to-beak or lip singularities^{4,5} (where the plane of focus touches a cusped edge of the caustic surface) or at swallowtail catastrophes (singular points of the caustic surface). Guided by these principles it was possible to devise a series of catastrophes (the line drawings on Fig. 5) to account for the transformations on the photographs. The line drawings show the patterns that would be formed in the limit of geometrical optics where the wavelength approaches zero. There are further singular points of caustic surfaces, associated with elliptic and hyperbolic umbilic catastrophes, but since these catastrophes are symmetric in their unfoldings they do not result in changes in the topology of the two-dimensional patterns; an elliptic umbilic occurs on Fig. 5b as the cusped 'triangle' shrinks to a point and expands again. All this behaviour is typical (generic): no special care is required in orienting the lollipops or in manufacturing them (other than making them smooth). Qualitatively identical phenomena were also observed in reflection.

The tilting of a lollipop provides two more control variables, θ_1 and θ_2 , say. By changing θ_1 it is possible to find a through-focus sequence where the cusped triangle shrinks to a point just as it reaches one of the caustic lines; this is a parabolic umbilic catastrophe point P on the three-dimensional caustic hypersurface in the four-dimensional control space C. The sequence on Fig. 5 constitutes a three-dimensional section S of C, and the fact that P lies close to S explains the small scale of detail in the patterns. In Thom's terminology⁴ the parabolic umbilic is the 'organising centre' for caustics near P and its unfolding justifies the sequence of patterns in the line drawings of Fig. 5. It is also possible to make two swallowtails coincide; this is a butterfly catastrophe. Finally, by also changing θ_2 this butterfly and parabolic umbilic can be made to coincide at one stage in the through-focus sequence; the two-dimensional pattern (Fig. 6) is then a singular section of

the higher-order catastrophe, presumably involving five control variables, which organises all the triple junction caustic patterns discussed here.

We draw two conclusions from this study. First, nature's line patterns are not all of the same sort; the triple junctions generic in mud cracks cannot occur with caustics. Second, the geometrical optics of cylindrically symmetric artefacts such as telescopes, where departures from the ideal point focus are treated as 'aberrations', is very different from the geometrical optics of nature, where the generic forms of caustic surfaces are governed by the mathematics of catastrophe theory.

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M. V. BERRY
J. F. NYE

H. H. Wills Physics Laboratory,
Tyndall Avenue,
Bristol, UK

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Polarised infrared cathodoluminescence from platelet defects in natural diamonds

We report here that the large platelet defects occasionally found in natural diamonds emit polarised cathodoluminescence in the near infrared. Lattice defects in natural diamonds attract as much scientific enquiry today as at any time since the anomalous 'spike' diffuse X-ray reflections from single-crystal diamonds were discovered¹. That platelet-type defects segregated on {100} planes of the diamond matrix could well account for the 'spike' X-ray reflection observations^{1,2} was shown by Frank³. Transmission microscopy⁴ revealed just such platelets, with diameters generally in the range 10 to 100 nm, profusely present within the most common form of diamond (Type Ia). Mendelssohn⁵ described a specimen containing platelets on {100} that were more than two orders of magnitude greater in diameter than those that had become well known through transmission electron microscopy. The large platelets emitted a green-yellow cathodoluminescence which made them conspicuous when they lay not deeper below the specimen surface than the penetration range of the bombarding electron beam (~10 μ m). Work in our laboratory has shown that platelets on {100} span the gamut of sizes from the largest observed by Mendelssohn down to the more familiar sub-micrometre objects seen electron-microscopically. This knowledge has been acquired by combining X-ray topography (using both normal Bragg reflections and the anomalous 'spike' reflections), cathodoluminescence topography, ultraviolet absorption topography and transmission electron microscopy at 100 kV and 1 MeV (refs 6,7). Platelets exceeding 5-10 μ m in maximum dimension are rare compared with those of sub-micrometre size; but our experience affords us a good chance of identifying solely by X-ray topography regions within diamonds where there reside platelets having extreme dimensions exceeding 1-2 μ m (and such platelets we dub 'giant').

We conclude⁸ that the principal visible emission from individual platelets is the 'H3' system^{8,9}; although in our experiments (so far done only at or slightly above room temperature) we have not yet succeeded in spectrographically recording the 2.46 eV zero-phonon line in the weak spectra of single platelets. The polarisation of the visible cathodo-

luminescence from platelets⁶ is notable: the measured ratio of intensity emitted with **E** vector in the platelet plane to that with **E** vector normal to the platelet being about 10.

There is much uncertainty regarding composition and structure of the platelets¹⁰⁻¹². Apart from expressing dissent with the view of Evans¹³ and Woods⁷ that the platelets are composed of interstitial carbon atoms, we will not debate this topic since our concern is to present new findings on the optical properties of platelets. The discovery that cathodoluminescence from giant platelets can be seen in the near infrared using an image converter has been briefly reported⁶. This observation has been followed up by photographic recording with Kodak High Speed Infrared film. It has now been discovered that the infrared emission from platelets is polarised with **E** vector in the platelet plane, but with a considerably higher polarisation ratio than in the case of their visible emissions.

The cathodoluminescence topographs in Fig. 1 (taken by means similar to those previously described¹⁴) show part of the specimen C19 studied by Mendelssohn. Here platelets range up to 40 μm in extreme dimensions. Differing brightnesses of platelets which lie in the cube plane nearly parallel to the specimen surface result from their differences in depth below the surface; these images are unaffected by rotation of the Polaroid filter. (In this specimen fan-shapes and kite-shapes predominate among the largest platelets; in other specimens elongated shapes aligned randomly along one or other of the two $\langle 110 \rangle$ axes in the platelet plane are more common.) The appreciable width of edge-on platelet images arises from shortcomings of the optical system which was not designed for infrared use. Contrast between platelets and matrix is far superior to that in the visible region where platelet emission has to compete with the low-energy tail of the cathodoluminescence continuum known as Band A (refs 9, 15). To facilitate comparison of features in Fig. 1, a Polaroid filter giving only partial extinction at infrared wavelengths was used. In experiments

undertaken to assess the degree of polarisation of the infrared emission we used Polaroid Type HR linear polariser which is highly effective at the longest wavelengths (about 900 nm) recordable by Kodak High Speed Infrared film. Despite a sixfold increase in electron beam current per unit area of specimen as well as reductions in optical magnification so as to maximise image brightness of the polarisation component attenuated by the Polaroid, we could determine only a lower limit, about 40, of the ratio of intensity of radiation with **E** vector parallel to the platelet plane to that with **E** vector normal to the platelet. Our results reinforce our earlier conclusion⁶ that we are observing the emission band peaking at 1.25 eV (990 nm) described by Wight *et al.*¹⁶ which correlates well with the infrared absorption at 170 meV (7.3 μm) generally accepted as a measure of total area of the common, sub-micrometre platelets in unit volume of specimen. The high degree of polarisation of platelet infrared emission constitutes a well-defined optical characteristic that any model for platelet structure (and for optical processes associated with platelets) must satisfactorily accommodate.

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I. KIFLAWI
A. R. LANG

H. H. Wills Physics Laboratory,
Royal Fort, Bristol, UK

Received 12 January; accepted 7 March 1977.

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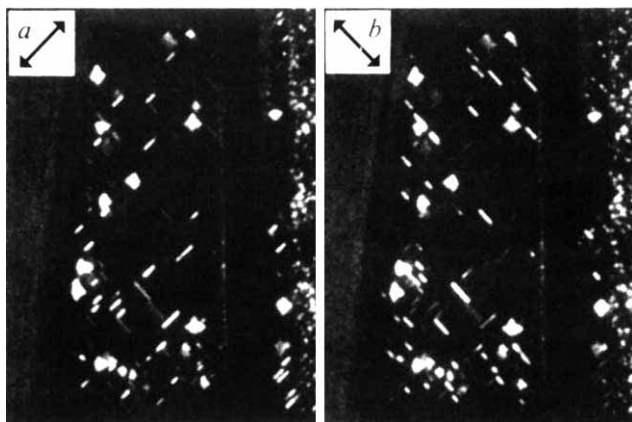


Fig. 1 Infrared cathodoluminescence from large platelets lying on cube planes in a natural diamond. The specimen surface is polished about 5° off (001) orientation: hence platelets close to the specimen surface and lying in (001) are seen nearly face-on. The direction [110] points horizontally to the right. Platelets parallel to (100) are seen edge-on as bright lines running from top right to lower left, and those parallel to (010) as bright lines running from top left to lower right. Field width is 0.5 mm; the length of the arrows indicating **E** vector directions is 100 μm . Electron beam energy 20 kV, specimen current 1.1 $\mu\text{A mm}^{-2}$. Photographed on Kodak High Speed Infrared film through the visually opaque, infrared transmitting Kodak-Wratten filter 87C and through a Polaroid filter used for visible wavelengths. The lighter areas and strips seen trending roughly vertically on either side of the field are due to numerous smaller platelets not fully resolved individually which reside in crystal growth layers parallel to (111) on the left and to (111) on the right. *a*, Polaroid rotated to transmit the **E** vector lying in the plane (100); *b*, lying in (010), as indicated by arrows.

Supported ^{242}Cm in the marine environment

BOWEN and Livingston¹ have reported the presence of ^{242}Cm (half-life 163 d) in two samples of sea water which had been kept in sealed containers for 12 yr since the time of collection. Because the ^{242}Cm originally present in the samples would have decayed completely after this period of storage, they suggested that the ^{242}Cm which they detect is supported by a long-lived precursor, $^{242\text{m}}\text{Am}$; this radionuclide would not itself be detectable in these samples because of its much longer half-life (152 yr). They invited other workers in radiochemistry to search for similar evidence for $^{242\text{m}}\text{Am}$ in the environment, and we have done so in some samples collected from the vicinity of the fuel-processing plant at Windscale where controlled discharges of transuranic nuclides to the marine environment are permitted under the provisions of the United Kingdom Radioactive Substances Act, 1960. The radiological implications of these discharges have been discussed by Hetherington *et al.*².

We obtained a sample on 15 December 1974 from the area of the processing plant and extracted the actinides from the sample matrix by strong acid digestion in oxidising conditions. After reduction with sulphur dioxide they were concentrated by coprecipitation, first on calcium oxalate and then on 5 mg of added neodymium carrier. Am, Cm and Nd were then separated from the Pu by anion exchange

in 9 M HCl and further purified by precipitation as fluoride in acid solution, followed by conversion to the hydroxide. Am and Cm were then separated from Nd by anion exchange in a 4 M NH_4SCN medium at 80 °C and electro-deposited on to a stainless steel disk from an $(\text{NH}_4)_2\text{SO}_4/\text{H}_2\text{SO}_4$ medium. ^{243}Am was used as a yield tracer.

The separated source of Am and Cm was first measured on 24 July 1975 by α -spectrometry and was then measured at intervals over a period of some 500 d to follow the decay of ^{242}Cm . The results are shown in Table 1.

Table 1 Decay of separated ^{242}Cm

Date	Decay period (d)	^{242}Cm (pCi)	^{241}Am (pCi)
24 July 1975	0	48.04 ± 1.79	43.1 ± 1.6
14 Jan. 1976	174	23.47 ± 0.36	—
17 May 1976	298	14.32 ± 0.27	—
12 Jan. 1977	538	5.07 ± 0.28	—

The $\pm$ figures denote σ error on counting only. Data are corrected for Am yield.

A regression on these data by the method of least squares produced a derived half-life value of 166.0 ± 2.1 (1σ) d, which is very close to the accepted value for ^{242}Cm of 163 d; the coefficient of determination (r^2) was 1.00. If the half-life was taken as 163 d, however, and the assumption then made that the apparent longer half-life was due to a small, constant amount of ^{242}Cm , supported by a precursor with very long half-life, then the decay equation could be restated

$$(y-d) = K \exp(-0.693/163)t$$

where y is measured activity (total) of ^{242}Cm (pCi), d is supported ^{242}Cm (pCi), t is time of decay (d) and K is unsupported ^{242}Cm at time zero (pCi).

This equation can be arranged

$$y = d + K \exp(-0.693/163)t$$

A linear regression can again be performed with $\exp(-0.693/163)t$ as the x -value and the measured ^{242}Cm activity as the y -value. The value of d (the equilibrium value of supported ^{242}Cm) obtained in this way was 0.60 ± 0.32 (1σ) pCi, compared with the ^{241}Am value of 43.1 ± 1.6 pCi. Thus the ^{242m}Am is $1.36 \pm 0.73\%$ of the ^{241}Am : this is significantly different from zero ($P=0.03$).

The analysis of other samples in the Windscale area has not included counting at intervals to measure decay of ^{242}Cm . But, following the procedure of Bowen and Livingston, Am and Cm were recently separated from some marine sediment samples collected in April 1973, some seven half-lives of ^{242}Cm after collection; in none of them did the total ^{242}Cm exceed 0.2% of the ^{241}Am , and the supported $^{242}\text{Cm}/^{241}\text{Am}$ in these samples can therefore be stated unequivocally to be $\leq 0.2\%$.

The results of the investigation indicate that little, if any, supported ^{242}Cm has been detected in this area but further work is in hand to check these preliminary data.

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J. W. R. DUTTON
M. B. LOVETT

Ministry of Agriculture, Fisheries and Food,
Fisheries Radiobiological Laboratory,
Hamilton Dock,
Lowestoft,
Suffolk, UK

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Are rare earth elements mobile during spilitisation?

FROM the study of rare earth elements (REE) in basalts, spilites and hydrothermally altered basalts in the ocean floor^{1–3}, ophiolites^{6,7}, geosynclines^{3,8} and low-grade to greenschist facies regions⁹ it has come to be accepted that the REE are immobile during rock alteration. This conclusion has led to the use of certain abundance ratios, particularly La/Sm and La/Yb, as indicators of parent magma type and genesis. Despite some suggestions^{2,10} that REE, such as La and Ce, may be mobile during sea-water/rock interaction, hydrothermal alteration or spilitisation of igneous rocks, the REE continue to be treated (e.g. refs 6 and 7) in an analogous fashion to Ti, Y and Zr (ref. 11). Here we show from analyses of a variably altered tholeiite flow that spilitisation may, in some circumstances, affect the REE abundances and their chondrite normalised pattern.

One major obstacle to the study of the relative stability of REE abundances during rock alteration is the rarity of outcrops that show a continuous gradation from a fresh to a highly altered condition. The Bhoiwada section, part of a volcanic sequence on Bombay Island, India, is such an occurrence and allows a test to be made of possible mobility of REE during spilitisation.

The Bhoiwada section exhibits^{12–15} the gradation of a fresh, black, plagioclase-clinopyroxene tholeiite into a green, albite-chlorite-zeolite spilitite within a single Deccan flow unit, 30 m thick (Fig. 1). Details of petrography, major and minor element analyses and field description are provided by Vallance¹⁴ and Sukheswala¹². Vallance¹⁴ also presents evidence to show that the section represents one flow unit, part of which may have been subaerial and part subaqueous. The section can be divided into five zones¹⁴. Only zone 5 is pillowed; we have subdivided an outer margin of a pillow into three parts (Fig. 1b). The pillows are often highly amygdaloidal and contain rosettes of laumontite, with prehnite, quartz and calcite. There is a concentration of amygdales immediately below the thin (less than 5 mm thick) black, chlorite-rich rind. Brief mineralogical descriptions for each sample are given with Fig. 2.

REE abundances in powdered rock samples were determined by instrumental neutron activation analysis¹⁶. Accuracy and precision are estimated to be about 4% at the 95% confidence level, for all REE except the Ho data which are about 10%.

REE abundances for the five zones are presented as chondrite normalised diagrams in Fig. 2 (a) and Fig. 3. La/Sm and La/Yb ratios are given in Table 1. For comparison, REE patterns are shown (Fig. 2) for two of Kuno's Japanese basalt types¹⁷, an alkali basalt and nepheline benmoreite from the Dunedin volcano, New Zealand¹⁸, and a tholeiite from the neovolcanic zone in

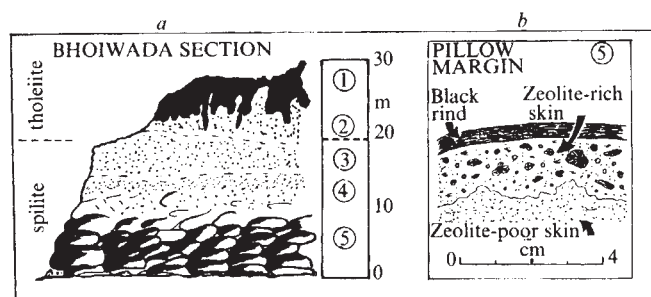


Fig. 1 a, Sketch of Bhoiwada section and its five zones (after Vallance¹⁴ and Sukheswala¹²); b, Sketch of pillow margin, zone 5.

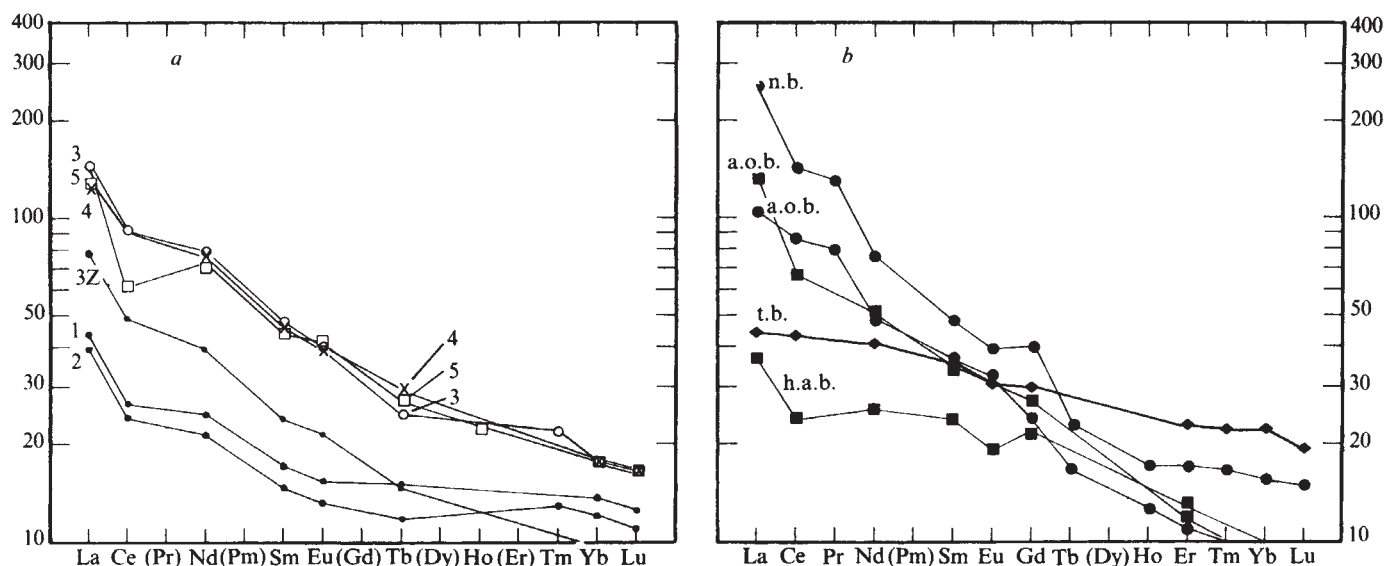


Fig. 2 REE abundances (chondrite normalised after Haskin *et al.*²⁴) from a, Bhoiwada, zones 1-5. Sample descriptions: 1, plagioclase-clinopyroxene-(magnetite) tholeiite. Some phenocrystal plagioclase and clinopyroxene. Intersertal patches of altered glass. 2, Same as 1 except for appearance of small chlorite-filled amygdaloids and chloritised matrix. 3, (open circles) Chlorite-albite-prehnite-spilite. 3Z: Strongly zeolitised spilite. Laumontite-prehnite-quartz amygdaloids within a chlorite-albite matrix. 4, (crosses) Chlorite amygdaloids within albite-chlorite-spilite. 5, (open squares) Core of pillow. Albite-chlorite-prehnite-spilite. Amygdaloids of chlorite and prehnite. All spilites possess relict clinopyroxenes. b Fresh recent lavas: ■, Japan—high-alumina basalt (h.a.b.) from Fuji volcano, Mishima and alkali olivine basalt (a.o.b.) from Oki Dogo Island¹⁷. These two basalts are representative of two of Kuno's basalt types. ●, New Zealand—alkali olivine basalt and nepheline benmoreite (n.b.) from the Dunedin volcano, East Otago¹⁸. ♦, Iceland—tholeiite basalt (t.b.) from Askja caldera, neovolcanic zone¹⁹.

Iceland¹⁹. La/Yb ratios are listed for several basalt types from different environments in Table 2.

The marked increase in the concentrations of REE with spiliteisation can be seen easily from Figs 2 and 3. Not only do absolute abundances change but also the La/Sm and La/Yb concentration ratios (Table 1). The observed

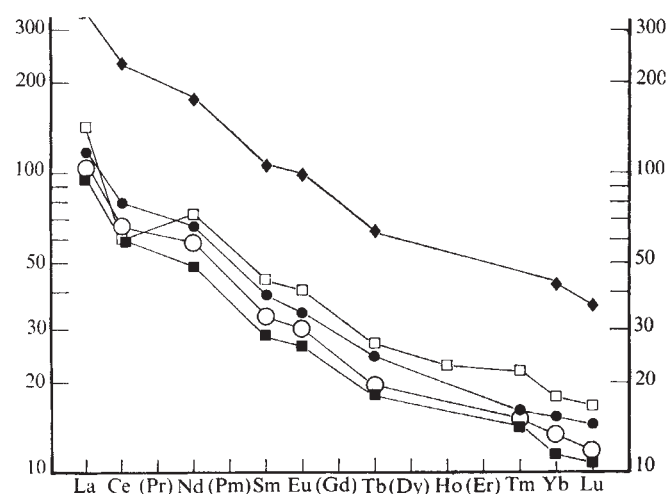


Fig. 3 Chondrite normalised REE abundances within a single pillow—zone 5 (see Fig. 1). ♦, black rind; □, core; ●, zeolite-free skin; ○, zeolite-rich skin; ■, 15087B—a pillow skin donated by Vallance.

changes are consistent with the generation of spilites as being a secondary process¹⁴⁻¹⁵. However, it is to be noted that there is virtually no change in the ratios of the values observed in slightly altered and more extensively altered material.

These results have implications for former studies in which REE abundance patterns of altered basalt and spilites were compared with those of fresh rocks. For example, the pattern of a zone 5 pillow alone might have been interpreted as the spiliteised equivalent of an alkali basalt (see Figs 2b and 3). The shift in La/Yb ratios

transcends values from numerous basalt types and geological environments (Tables 1 and 2). The variation also mimics natural basalt crystallisation-differentiation patterns. Consequently our results for the specific case of a light REE (LREE) enriched pattern at Bhoiwada indicate that it is unwise to interpret altered basalts as possessing alkaline affinities on the basis of the REE abundances alone.

Two examples of such interpretations are: the REE patterns shown by rocks of a lower Palaeozoic greenstone terrain in West Norway, indicate different degrees of partial melting of the source material for parent magma⁶; and the suggestion⁷ that the genesis of a Mesozoic ophiolite, exposed in central Greece, involved the existence of a tensional rift system. Our data show that an alternative feasible interpretation is that the REE patterns have resulted partly from secondary metamorphic or metasomatic activity. A similar interpretation¹⁰ has been made for some zeolitised Icelandic lavas.

The layer silicates, such as chlorite, and the low-birefringent, very fine-grained matrix in the spilites, could play a significant role in determining the REE abundance patterns. Roaldset and Rosenquist²⁰⁻²² describe chlorite in Quaternary clays of the Numedal area, Norway, as capable of increasing the La content by almost four times, through

Table 1 La/Sm and La/Yb weight ratio variation within the five zones of Bhoiwada section

Zone	La/Sm	La/Yb
1	4.7±0.2	5.4±0.2
2	4.9±0.2	5.5±0.2
3	5.9±0.3	14.0±0.6
3 (strongly zeolitised)	6.1±0.3	13.5±0.6
4	5.2±0.2	12.4±0.5
5	6.0±0.3	13.4±0.6
a, black rind	5.7±0.3	13.0±0.6
b, skin—zeolite rich	5.6±0.3	12.9±0.6
c, skin—zeolite poor	5.9±0.3	13.2±0.6
d, core	6.1±0.3	14.3±0.6
e, skin—15087B		

Table 2 Common La/Yb weight ratios from different environments

	La/Yb
Hawaiian tholeiitic series ¹	3.9–7.0
Iceland tholeiite ¹	2.7–7.2
DSDP Leg 15, site 15 ¹	6.2–7.5
Hawaiian alkali series ¹	8–15
Calc-alkaline extrusives ²³	6–8
Island arc tholeiites ²³	1–2

adsorption. Their studies also suggest that such adsorption processes raise significantly both the La/Sm and La/Yb ratios.

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PHILLIP L. HELLMAN

School of Earth Sciences,
Macquarie University,
North Ryde NSW 2113,
Australia

PAUL HENDERSON

Department of Mineralogy,
British Museum (Natural History)
Cromwell Road,
London, UK

Received 9 February; accepted 18 March 1977.

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540-Myr-old anorthosite complex in the Grenville Province of Quebec, Canada

MASSIF-TYPE anorthosites and related rocks are an important component of the Grenville and Nain structural provinces of the Canadian Shield (Fig. 1), as well as in high grade metamorphic terrains in other parts of the world. There are many outstanding problems in the study of these rocks, not least of which is their age. Most geochronological data have been obtained from the associated granitic rocks of this suite as anorthosite itself contains very little rubidium and zirconium. Because of post-crystallisation deformation, however, the relationship between the anorthosite and the

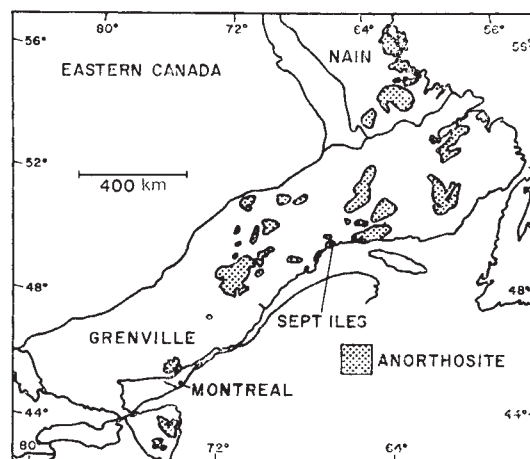


Fig. 1 The location of the Sept Iles and other major anorthosite complexes in the Grenville and Nain Provinces are shown. The northern boundary fault of the St Lawrence Graben follows the shore line in most of this area.

associated rocks is often tenuous and frequently disputed. Within the Grenville Province metamorphosed rocks believed to be genetically associated with anorthosites have yielded ages ranging from 1,020 to 1,130 Myr (refs 1–4). The high-grade of metamorphism surrounding the Morin and Lac St Jean anorthosites is believed by some researchers to be due to anorthosite emplacement. These 'aureoles' yield Rb–Sr ages^{5,6} of about 1,500 Myr. Anorthosite complexes in the Nain Province are undeformed, and the adamellite of the suite has yielded a U–Pb zircon age⁷ of about 1,300 Myr. Barton⁸ obtained a Rb–Sr isochron age of 1,418 Myr from a digested inclusion in the Nain anorthosite. Here we present geochronological data from the undeformed Sept Iles anorthosite, and show it to be Cambrian in age.

The Sept Iles anorthosite complex is situated on the north shore of the Gulf of St Lawrence, about 800 km north-east of Montreal, on the southern edge of the exposed portion of the Grenville Province. Apart from rare alkaline rocks, ages of Grenville Province rocks exceed 900 Myr. The Sept Iles anorthosite complex is, however, situated on the boundary between the St Lawrence Graben and the Grenville Province, which is the locus of intrusion of alkaline rocks ranging in age from early Cambrian to Cretaceous⁹. Its boundaries are clearly indicated on aeromagnetic maps, and by a substantial positive gravity anomaly measuring approximately 50 km × 70 km. Its host rocks on shore are thought to be a moderately deformed anorthosite. The complex has been considered to be a good example of undeformed Grenville anorthosite¹⁰. The islands have been mapped in detail (S. T. Ahmedali, personal communication) and consist of anorthosite grading upward into well layered gabbro. The gabbro is overlain along a sub-horizontal contact near sea level by granitic rocks ranging from partly layered monzonite near the base to adamellite. The granitic rocks are slightly alkaline in their chemistry and contain alkali pyroxenes and amphiboles. Field relationships show clearly that the more basic members of the suite were only partly consolidated during the emplacement of the monzonite. An age from this unit would therefore date the anorthosite. Verification of this relationship was obtained by isotopic means from more rubidium-rich segregations within the anorthosite itself.

Isochrons have been obtained from a single outcrop of the layered monzonite and from a 500-m section through the adamellite. These yield ages of 538 ± 17 Myr and 552 ± 12 Myr (Fig. 2a, b) (all errors are expressed as single standard deviations). Rb–Sr data have also been obtained

from alkali rich patches within the anorthosite. The contemporaneity of these bodies and the anorthosite is clearly visible in the field, because of the lack of deformation (Fig. 3). The host anorthosite consists of black plagioclase crystals 1 to 4 cm in length, with small amounts of interstitial mafic material. As the pegmatite is approached the grain size of the plagioclase increases, and these large crystals project into the zone of micrographic orthoclase and quartz. These patches are less than 1 m in diameter and are frequently aligned along what are assumed to have been zones of low pressure in the semiconsolidated anorthosite magma. More conventional anorthosite pegmatites also occur in zones between the orthoclase rich areas.

The isochron of Fig. 2c is from two adjacent pegmatites and their host anorthosites. The points define an age of 555 ± 12 Myr and an initial ratio of 0.7036 ± 0.0004 . The second isochron (Fig. 2d) is derived from three samples of a single pegmatite, combined with a single sample from a

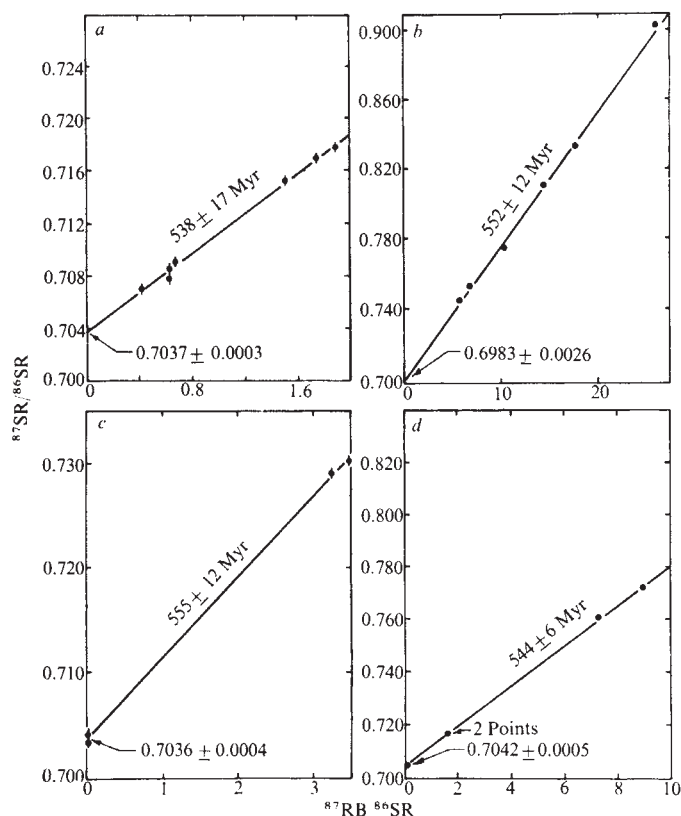


Fig. 2 Isochrons from the Sept Iles anorthosite and related rocks. The low initial ratio of (b) could be due to the abundance of xenoliths in this unit. Error bars (single s.d.) were calculated for both variables but are only shown where they exceed the diameter of the symbol. Ages were calculated using $\lambda^{87}\text{Rb} = 1.39 \times 10^{-11} \text{ yr}^{-1}$. a, Monzonite; b, adamellite; c, two adjacent pegmatites; d, three samples of a single pegmatite, combined with a single sample from a nearby pegmatite and the host anorthosite.

nearby pegmatite and the host anorthosite. The points are within experimental error of a line representing an age of 544 ± 6 Myr and an initial ratio of 0.7042 ± 0.0005 .

The initial ratios of these isochrons are consistent with ratios of 0.7039 (s.d. 0.0005) obtained from eight anorthosite and gabbro samples distant from the rare pegmatitic areas.

All ages reported here agree within narrow limits of experimental error, supporting the temporal relationships observed in the field. Granite, syenite, and carbonatite

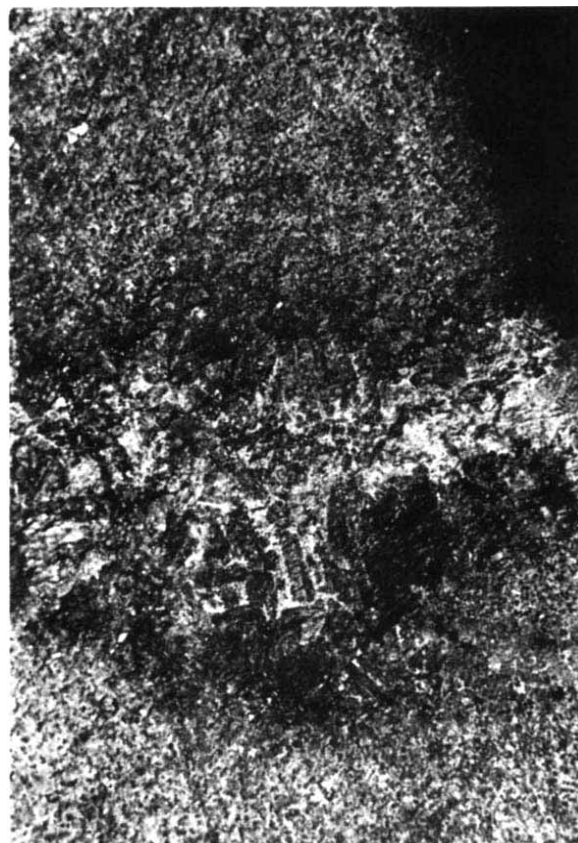


Fig. 3 The alkali-rich pegmatite shown here is located near the pegmatites used to construct isochron 2c. The large black plagioclase crystals that project into the micrographic zone are clearly visible.

bodies associated with the St Lawrence Graben system yield similar ages^{9,11}. The adamellite of the Sept Iles complex is very similar to some of these rift-related rocks. The location of anorthosite in a rift environment, associated with acidic alkaline rocks, may provide insight into the origin of both these suites. The results also suggest that many anorthosites may be anorogenic, although some may be deformed by later orogenic events.

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MICHAEL D. HIGGINS
RONALD DOIG

Department of Geological Sciences,
McGill University,
3450 University Street,
Montreal, Quebec H3A 2A7, Canada

Received 12 November 1976; accepted 1 March 1977.

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Phenology of sound-producing arctiid moths and the activity of insectivorous bats

THE lepidopteran family Arctiidae contains a number of species that emit high frequency sounds when tactually stimulated or exposed to the intense hunting cries of insectivorous bats¹⁻³. The sounds are produced as rapidly repeated clicks generated by the buckling action of the row of striae⁴ or microtymbals⁵ on the surface of the modified metepisternum (tymbal)⁶. The sounds seem to act as aposematic cues to discourage nocturnal, ultrasonically sensitive predators such as bats³. Flying bats will avoid otherwise palatable prey (mealworms) when accompanied by the recorded sounds of arctiid moths². If bats play an important role in the evolution of sound production in arctiids then bat activity levels in an area should influence the phenology of acoustically active arctiids there. By examining the incidence of some silent and sound-producing Nearctic arctiids and its relationship to ambient levels of bat activity, some of those influences can be demonstrated.

During the summers of 1975 and 1976, I collected moths from four ultraviolet lights located at the Queen's University Biology station near Chaffey's Locks, Leeds County, Ontario, Canada. The lights were inspected twice a night and any arctiids captured were immediately identified and tested for acoustic activity using a Brüel and Kjaer quarter-inch condenser microphone (type 4135) and measuring amplifier (type 2606) with a Tektronix 5103N storage oscilloscope⁷. Bat activity was monitored at the same location using an automated ultrasonic sensing system with detectors tuned to 40 kHz (ref. 8) directed over a lake where bats commonly fed. This location ruled out the possibility of detecting other sources of biological ultrasound (for example, insects).

Of the 24 arctiid species captured at the lights, 17 produced sound under light to heavy tactile stimulation (Table 1). Although the sounds generated by the moths were

extremely variable in amplitude-time patterning, frequency and intensity^{7,9} the degree of tactile stimulation required to elicit sound production was relatively constant within the categories listed in Table 1 for each species. A trend which soon became obvious was the timing of the emergence of silent and sound-producing species. During both summers, it was the silent species that appeared in the spring, and it was not until mid-July that a substantial proportion of moths caught at the lights were sound-producing species.

A comparison of the ambient bat activity levels in the area with the incidence of sound-producing arctiids (Fig. 1) reveals a close relationship between the two observations. A closer look at two genera lends insight into this relationship.

Phragmatobia (Stephens) includes two similar species that were captured at the study site: *P. assimilans* (Walker) and *P. rubicosa* (Harris). *P. assimilans* is a spring moth emerging early in May and disappearing before June. *P. rubicosa*, on the other hand, is a summer species, emerging in late July and remaining until August. *P. rubicosa* is an acoustically active species and will emit sounds under moderate tactile stimulation; its congeneric, *P. assimilans*, however, is silent. Scanning electron micrographs of the tymbal surfaces (Fig. 2) reveal that both species possess microtymbals but the band appears less defined in *P. assimilans* and may be vestigial.

The two species of the genus, *Hypoprepia* (Hübner), *H. fucosa* (Hübner) and *H. miniata* (Kirby) are also superficially similar in size and coloration¹⁰ and are also separated in their emergence times. *H. fucosa* appears from July to mid-August, whereas *H. miniata* appears somewhat later. Both species in this genus, however, emerge during periods of high bat activity and produce sound when lightly stimulated.

Animals exposed to unique forms of predation may develop predator-specific defences¹¹ that act directly on the particular characteristic of the relevant predator. Thus, the high frequency emissions of certain arctiids may be

Table 1 Acoustic activity of arctiids surveyed in chronological order of emergence

Species	n	Degree of stimulation required to elicit sound§	Earliest date of collection	Time of emergence Latest date of collection
<i>P. assimilans</i> (Walker)	8*	Silent species	3/05/75	14/05/75
<i>Spilosoma virginica</i> (Fabricius)	20*	Silent species	13/05/75	22/07/75
			1/08/75	24/08/75‡
<i>S. prima</i> (Slosson)	4†	Silent species	13/05/75	05/06/75
<i>S. congrua</i> (Walker)	5†	Silent species	13/05/75	05/06/75
<i>Halysidota caryae</i> (Harris)	10*	Silent species	29/05/75	14/06/75
<i>Hyphantria cunea</i> (Drury)	15*	Silent species	10/06/75	18/06/75
<i>Callarctia virguncula</i> (Kirby)	3*	Heavy	15/06/75	22/06/75
<i>Pyrharctia isabella</i> (Smith)	25*	Moderate	16/06/75	25/06/75
				01/08/75‡
<i>Halysidota tessellaris</i> (Smith and Abbot)	35*	Moderate	15/06/75	30/07/75
<i>Cynia tenera</i> (Hübner)	30*	Light	17/06/75	07/07/75
<i>Callarctia anna</i> (Grote)	5*	Heavy		15/06/75
<i>Euchaetias oregonensis</i> (Strech)	9†	Light	14/06/75	16/07/76
<i>Estigmene acrea</i> (Drury)	5*	Silent species		07/07/75
<i>Hypoprepia fucosa</i> (Hübner)	30*	Light	07/07/75	08/08/75
<i>Callarctia virgo</i> (Linne)	3†	Heavy	07/07/75	18/08/75
<i>Euchaetias egle</i> (Drury)	5†	Light	17/07/75	28/07/75
<i>Holomelina aurantiaca</i> (Hübner)	7†	Moderate	17/07/75	18/08/75
<i>Haploa confusa</i> (Lyman)	4†	Heavy		17/07/75
<i>Haploa contigua</i> (Walker)	3†	Heavy		17/07/75
<i>Phragmatobia rubicosa</i> (Harris)	6†	Moderate	26/07/75	03/08/75
<i>Hypoprepia miniata</i> (Kirby)	6†	Light	26/07/75	24/08/75
<i>Lycomorpha pholus</i> (Drury)	2†	Light		28/07/75
<i>Crambidia pallida</i> (Packard)	5†	Light		01/08/75
<i>Callarctia arge</i> (Drury)	2†	Heavy		03/08/75

*Both sexes were tested and showed the same acoustical activity.

†Both sexes were not tested.

‡Second brood.

§Degrees of stimulation similar to Blest¹: light involves merely touching the moth; moderate indicates the moth had to be shaken or prodded; and heavy indicates that very severe handling was required.

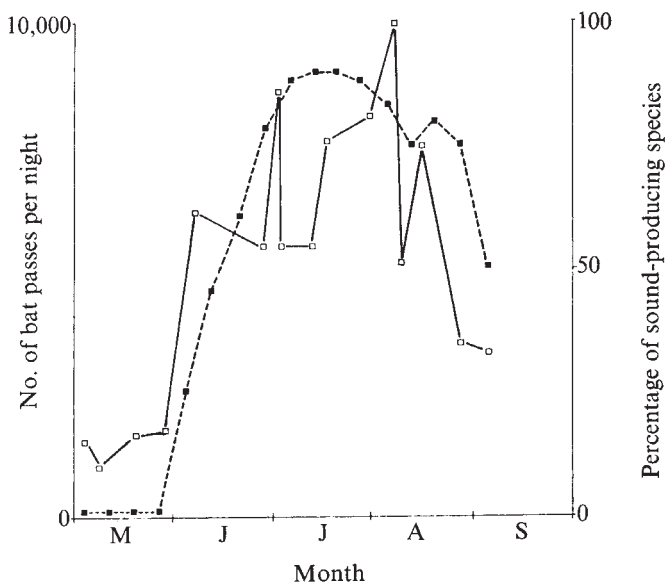


Fig. 1 Relationship between summer activity levels of insectivorous bats and phenology of sound-producing arctiid moth species. Bat activity (□) is recorded as 'bat passes'; incidence of acoustically active arctiids (■) are the averages per quarter month.

signals intended for the ultrasonically sensitive ears of predators such as bats. Since the maintenance of such specific defences should be dependent on the level of threat posed by the predators concerned, species exposed to lower levels of activity of these predators should not possess the defences of individuals more severely threatened. Therefore, the high proportion of silent arctiids present in the spring may be a response to the lower levels of bat activity at these times.

Phragmatobia offers an intriguing example of this relationship. *P. assimilans* is not only a silent species, similar to its acoustically active summer relative, *P. rubicosa*, but also possesses a tymbal structure which suggests that *P. assimilans* may have been a sound-producing species at one time. By shifting its emergence time to spring and thus avoiding the time of high bat activity (although this may not have been the original reason for the shift), *P. assimilans* no longer required a functional tymbal and now retains only a vestigial form of the organ. *Hypoprepia*

illustrates a similar situation, the important difference being that both species emerge during high levels of bat activity and both produce sound.

Silent species that persist into or emerge in mid-summer (for example, *Spilosoma virginica* (Fabricius) and *Estigmene acrea* (Drury)) may be less vulnerable to bats for a variety of reasons. *E. acrea* is a very large arctiid and this may afford it sufficient protection from most hunting bats. Since arctiids will perform aerial evasive tactics in the presence of echo-locating bats¹², there may be a greater adeptness at this and other anti-bat defences that have allowed certain silent arctiids to exist in times of high bat activity.

As bats are not the only ultrasonically sensitive insectivorous predators that arctiids must contend with, it is likely that the ambient summer levels of non-Chiropterans (for example, *Peromyscus*) may also exert an influence on the phenology of sound-producing arctiid species. The diverse interactions that arctiids have with their predators are poorly understood and must be clarified before some of the puzzling problems associated with comprehending this unique form of defence can be dealt with.

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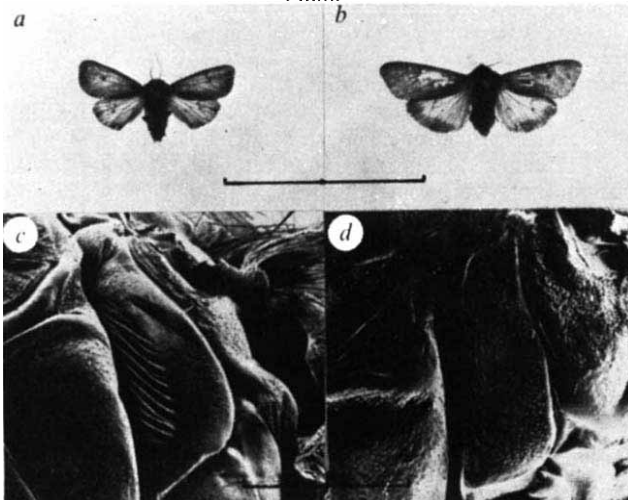
JAMES H. FULLARD

Department of Biology,
Carleton University,
Ottawa K1S 5B6, Canada

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Fig. 2 a and c, Acoustically active summer arctiid, *P. rubicosa*, and its tymbal. b and d, Silent spring species, *P. assimilans*, and its non-functional tymbal with what appears to be a vestigial micro-tymbal band. Upper scale indicates 40 mm; lower scale indicates 1 mm.



Survival value of fever in fish

THE phenomenon of behavioural fever, manifested as an increase in preferred temperature following injection of live or killed bacteria, or other pyrogenic substances, has been demonstrated in bony fishes¹⁻³, amphibians^{4,5}, reptiles⁶⁻¹⁰, and mammals^{11,12}. Ectothermic vertebrates, including some newborn mammals¹¹, are unable to increase their body temperatures much above ambient by physiological means, and so are largely limited to behavioural thermoregulation by selecting favourable temperatures in their environment. The behavioural febrile response of a lizard, *Dipsosaurus dorsalis*, has been shown to have adaptive value^{5,8-10} in conferring increased survival during infection by the Gram-negative bacterium *Aeromonas hydrophila*. *A. hydrophila* is pathogenic to bony fishes, amphibians and reptiles, causing haemorrhagic septicaemia. All three classes show similar (1-5 °C) elevations in preferred temperature in response to injection of this bacterium¹⁻¹⁰. The absolute preferred temperatures, however, both normal and febrile, differ considerably among these vertebrates. The preferred temperatures of lizards such as *D. dorsalis* (about 40 °C) are quickly lethal to most fishes and amphibians. We report here that fever significantly

enhances survival of goldfish, *Carassius auratus*, at febrile temperatures about 10 °C lower than those of *D. dorsalis*, after injection with live *A. hydrophila*.

To study the thermoregulatory behaviour of fish we used an electronic shuttlebox device which allows a fish to control water temperatures and, thereby, its body temperature, by its movements between chambers monitored by photocells^{13,14}. The behaviour of a fish in this device is equivalent to seeking out preferred temperatures in its natural environment¹⁵. At least some fishes have been shown to exhibit circadian changes in preferred temperature^{14,15}. Specifically, the goldfish exhibits a diel rhythm of preferred temperature with a pre-dawn peak (W. W. R., M. E. Casterlin, J. K. Matthey, S. T. Millington and A. C. Ostrowski, in preparation). Accordingly, for this study we pooled data for 24-h periods¹⁻⁴, to simplify presentation of the data by showing changes in mean preferred temperature for 24 h before and after injection with bacteria.

Earlier work with largemouth blackbass (*Micropterus salmoides*) and bluegill sunfish (*Lepomis macrochirus*) indicated a mean febrile increase in preferred temperature of 2.6 °C after injection with killed *A. hydrophila*¹. We therefore used a nominal febrile elevation of 2.5 °C to represent a typical fever in fish. We tested 10 goldfish in our thermoregulatory apparatus (Ichthyotron)¹³. These had a mean baseline (afebrile) preferred temperature of 27.9 °C, which agrees well with a previously published final preferendum value of 28.1 °C for *C. auratus*^{16,17}. Using a nominal 28 °C baseline temperature and the nominal 2.5 °C febrile elevation value, we tested three groups of 25 goldfish at fixed temperatures of 25.5 °C, 28.0 °C and 30.5 °C for 72 h. These represented, respectively, hypothermic, normothermic and febrile temperatures. The 75 goldfish, weighing about 4 g each, were injected intraperitoneally with 0.1 ml of sterile pyrogen-free fish saline containing 2×10^6 live *A. hydrophila* cells.

Goldfish maintained at a febrile temperature of 30.5 °C (Fig. 1) had the highest survival rate (84%), while only 64% survived at 28.0 °C and 24% at 25.5 °C. A control group of 10 fish, injected only with sterile saline, all survived at 25.5 °C. Another 10 fish injected with live *A. hydrophila* and allowed to thermoregulate in the shuttlebox attained an unexpectedly high mean febrile preferendum of 32.7 °C, a mean increase of 4.8 °C. The circadian rhythm of preferred temperature was maintained around the higher set-point. The high 4.8 °C febrile increase may have been due to multiplication of the live bacteria, resulting in a higher weight-specific dosage in these goldfish, which were smaller than the bluegills and bass tested previously¹ with killed bacteria. It may be relevant that the ultimate upper

incipient lethal temperature of goldfish is unusually high¹⁸ (about 41 °C) for fish—about 6 °C higher than that of bluegill and bass. None of the 10 febrile thermoregulating goldfish died, so the 32.7 °C temperature afforded even greater protection (Fig. 1) than did the nominal febrile temperature of 30.5 °C.

To examine the effect of temperature on growth rate *in vitro* of *A. hydrophila*, we grew cultures at 28, 30 and 33 °C (Fig. 2). We determined growth curves by inoculating 100 ml of glucose nutrient broth or brain-heart infusion broth with 0.1 ml of a 14 h broth culture; at 1–2 h intervals the cultures were plated and turbidometric readings made. There was no significant difference among the growth curves at the three test temperatures (Fig. 2), so the survival value of the fever apparently cannot be attributed to an effect of temperature on bacterial growth within the range of temperatures selected by goldfish. This is also true for lizards (*D. dorsalis*) at still higher temperatures⁸.

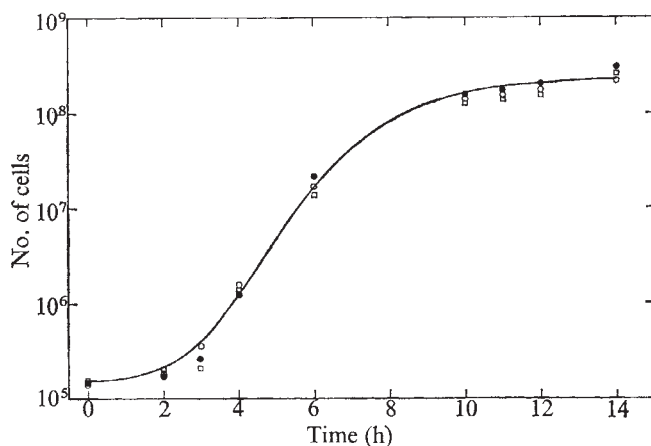


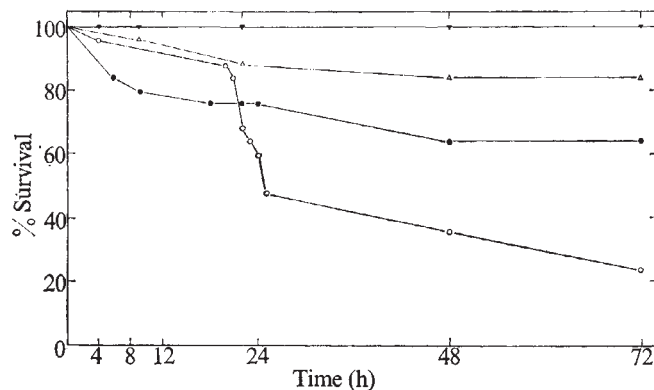
Fig. 2 Growth curves for three *A. hydrophila* cultures *in vitro* at 28 °C (□), 30 °C (○) and 33 °C (●).

Immunological responses of fishes and other poikilothermic animals have been shown to be significantly affected by temperature^{18,19}. The critical temperature levels are species-specific¹⁸, and vary considerably among classes¹⁹. For example, the normal body temperature of the lizard *D. dorsalis*⁵⁻¹⁰ exceeds even the febrile temperatures of the fish species studied, and indeed would be lethal to most fishes and amphibians. It thus seems that the absolute febrile temperature *per se* is less important than the febrile elevation above temperatures normal for the species.

Similar enhancement of survival during a viral disease by elevation of the water temperature has been reported for sockeye salmon, *Oncorhynchus nerka*²⁰. Mortality was prevented at 18 °C, which is 3.5 °C above the 14.5 °C normal preferred temperature of this cold-water salmonid species¹⁷. Amend²⁰ did not study behavioural responses to the virus infection. The virus was not destroyed by the 18 °C temperature, and enhancement of some critical hormone or enzyme activity was postulated as the cause of the increased survival²⁰. Another possibility is that increased phagocytosis could result from increased vascular permeability leading to greater mobility of the antigen-processing cells in the early stages of infection. It has also been suggested²¹ that there may be a relationship between temperature-dependent toxic effects of various substances and their effects on preferred temperatures of fishes, which might serve to reduce mortality.

Survival of infection can apparently be enhanced by fairly short-term increases in temperature, as the heliothermic *D. dorsalis* is able to thermoregulate only during the day, with body temperatures dropping to ambient

Fig. 1 Survival curves for goldfish at four temperatures after intraperitoneal injection with live *A. hydrophila*. Three groups of 25 fish each were held at fixed temperatures of 25.5 °C (○), 28.0 °C (●), and 30.5 °C (△); 10 fish (▼) were allowed to behaviourally thermoregulate at a mean temperature of 32.7 °C.



(12 °C) at night¹⁰; the daytime elevation of body temperature is apparently sufficient to enhance survival of infection⁸⁻¹⁰. Similarly, our febrile infected goldfish experienced no mortality following removal to holding tanks at room temperature (23 °C) after thermoregulating for several days at elevated temperatures. Avtalion¹⁸ has shown that after fish are allowed to develop a positive immunological memory at a high temperature, they are then able to produce antibodies even at low temperatures. It thus seems likely that some initial step in the immune response is enhanced by a febrile increase above the normal preferred temperature of a species, and that this is effective even if the febrile temperature is not constantly or indefinitely maintained.

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JERRY B. COVERT

Department of Biology,
The Pennsylvania State University
Hazleton, Pennsylvania 18201

WILLIAM W. REYNOLDS

Department of Biology,
The Pennsylvania State University,
Wilkes-Barre, Pennsylvania 18708

Received 14 January; accepted 1 March 1977.

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Soluble fragments from fungal cell walls elicit defence reactions in crayfish

ALTHOUGH most fungi that parasitise arthropods are relatively harmless, many can penetrate different types of cuticle¹. Thus active defence mechanisms presumably operate *in vivo*. In crayfish, for example, resistance to fungal penetration is associated with melanisation of the cell wall of the fungus by host phenols and phenoloxidase². This constitutes a system for recognising the invading organism at different levels, from the cuticle surface inwards³. Melanisation in insect blood, due to phenoloxidase activity, is also induced by the presence of cells or cell walls of fungi^{4,5}. Crayfish phenoloxidase in cuticle and blood is highly and specifically activated in contact with the purified hyphal cell walls of most fungi but not with other plant cell walls⁶. We have now found that soluble β -1,3-glucans from fungal cell walls activate the enzyme and may serve as specific elicitors of recognition in crayfish of invading fungal parasites.

Haemolymph was bled from the abdominal haemocoel of crayfish, *Astacus astacus*, using a hypodermic needle (0.8 mm diameter, 21 gauge). Because phenoloxidase is released by blood cells during clotting, cell free serum from pooled, clotted blood was used as a source of enzyme⁶. The clot was homogenised and centrifuged for 10 min at 3,000g. The phenoloxidase activity of the serum obtained was determined in a mixture of 0.2 ml serum, 0.1 ml dihydroxy-

phenyl alanine (dopa, 4 g l⁻¹), and 0.2 ml 0.01 M sodium acetate buffer, pH 5.2, with or without dissolved cell wall extract. The mixtures were incubated at room temperature, about 22 °C, in an open microscope slide cuvette⁷ for about 30 min. Before addition to the serum mixture the cell wall extract was preheated to 100 °C for 20 min to disperse aggregates. After covering the slide cuvette with a cover-slip the activity of phenoloxidase on dopa was calculated from the increase in absorbance at 480 nm using a Shimadzu MPS-50 L photometer⁷.

Zymosan (purified cell walls from *Saccharomyces cerevisiae*) activated crayfish phenoloxidase, especially in regions close to the cell wall surface (Fig. 1). This effect is similar to that seen in fungal cell walls purified in alcoholic KOH⁶. Furthermore, the supernatant from centrifuged suspensions of Zymosan or purified cell walls (even after six washings) of different fungi strongly activated phenoloxidase. Apparently, water-soluble fragments with activating capacity were released in very small concentrations from the solid cell walls.

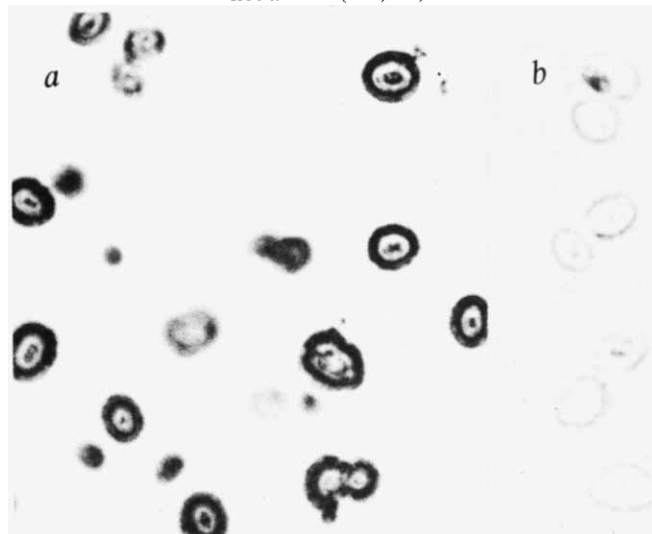
In the present work we used the supernatant (containing the yeast cell wall activator) from a 1% suspension of Zymosan suspended in 0.01 M sodium acetate, pH 5.2. The molecular weight of the activator was estimated on a column (26 mm × 550 mm) of Sephadex G 200, using acetate buffer at pH 5.2. Blue dextran 2000 (Pharmacia), bovine γ globulin and trypsin (both Sigma) were used as standards. When compared with the standards, the apparent molecular weight of the activator was between 10⁵ and 10⁶; small amounts of material with a molecular weight between 3 × 10⁴ and 10⁵ were also present. It seems that the elicitor is not a distinct molecule but consists of macromolecules or molecular aggregates of varying size.

Most elicitor activity was not bound to a cation (SP-Sephadex C 25), nor to an anion exchange resin (DEAE-Sephadex A 25) when the material was dissolved in and eluted with 0.01 M sodium acetate buffer, pH 5.2, and 0.01 M Tris-HCl buffer, pH 8.2, respectively and was apparently not charged to any detectable extent at these pH values.

The activator solution (from 1% Zymosan) contained about 0.4 g of dry material per l (desiccation at 100 °C for 3 h). It activated phenoloxidase even when diluted 10,000-fold. Elicitor activity could therefore be measured at least down to a concentration of about 10⁻¹¹ M.

The yeast cell wall elicitor was also pretreated at 22 °C for 1-24 h with different hydrolytic enzymes (0.2 mg ml⁻¹) to investigate the possibility that the elicitor molecules

Fig. 1 Zymosan activation of phenoloxidase of the cell free serum of crayfish. In the presence of a substrate, dopa, the cell walls thereby become melanised (dark). a, Dopa added; b, dopa not added. (×1,000).



could be inactivated (Table 1). The samples were heated (100 °C, 20 min) to destroy lytic enzyme activity and disperse aggregates before addition to the test mixture containing serum and dopa. Results were compared with the corresponding heated control sample containing the hydrolytic enzyme alone. As *Saccharomyces* cell walls contain mostly β -1,3-glucans⁸, it was not surprising that endo- β -1,3-glucanase affected the soluble cell wall fragments. This enzyme increased the activating capacity of the cell wall fragments (Table 1), especially after short exposure. Exposure for several days, however, did not destroy the activator substance or significantly decrease its molecular weight. Owing to the enzyme activity the number of active groups on the glucans was probably increased by exposing new, activating groups derived from the splitting of the large molecules. Digestion of the soluble fraction from 1% Zymosan suspension for 0.5 and 3 h with endo-glucanase followed by vacuum dialysis showed that none of the active molecules formed by hydrolysis had a molecular weight less than 20,000.

After long treatment with endoglucanase (Table 1) the additional, activating groups, but only these, were apparently digested and destroyed by the enzyme. Exo- β -1,3-glucanase, however, almost completely destroyed all the activating capacity of the glucans, suggesting that chains of β -1,3-linked glucose residues are involved.

Proteases had little or no effect and did not change the molecular weight of the bulk of the activator. Treatment with cellulase increased the activating effect considerably (Table 1). Traces of β -1,3-glucanase activity may be responsible for this, unless cellulosic components were also present in the glucans.

Since β -1,3-glucans seem to be involved in the activation, laminaran, a sparsely branched β -1,3-, β -1,6-glucan (from the alga *Laminaria hyperborea*) with an overall degree of polymerisation of about 30 glucose units, was also tested against the crayfish serum. At concentrations of 4×10^{-4} to 2×10^{-5} M this compound strongly inhibited phenoloxidase activity but at 4×10^{-6} to 10^{-6} M it was stimulatory. Activation could be detected down to about 10^{-7} M. The reason for the inhibitory effect at the higher concentrations is not yet known.

Pretreatment of 0.1% laminaran for 24 h with endo- or exo- β -1,3-glucanase destroyed activating capacity (Table 1).

Table 1 Effect of soluble *Saccharomyces* cell wall fragments and of laminaran on crayfish phenoloxidase after pretreatment with lytic enzymes

Pretreatment	Increase in activity of phenoloxidase* (%)	
	Zymosan	Laminaran
None	600 (0.66)†	160 (0.18)
Pronase	600 (0.66)	—
Trypsin	650 (0.72)	—
Cellulase	900 (0.99)	—
Endo- β -1,3-glucanase	800† (0.88)	0 (0)
Exo- β -1,3-glucanase	60 (0.07)	0 (0)

Pretreatment of 1 mg ml⁻¹ laminaran (Koch-Light) and of the soluble fraction (0.4 mg substance per ml) from a 1% suspension of purified *S. cerevisiae* cell walls (Zymosan, Koch-Light) was carried out at pH 5.2 in 0.01 M sodium acetate buffer for 24 h. Lytic enzymes used (0.2 mg ml⁻¹) were: Pronase (Sigma), trypsin (Difco), cellulase (S173, from *Trichoderma viride*, having traces of β -1,3-glucanase activity) endo- β -1,3-glucanase (S176G, from *Rhizopus arrhizus*) and exo- β -1,3-glucanase (highly purified). Phenoloxidase activity against dopa was measured photometrically. Percentage increase in activity after addition of tenfold diluted, activating cell wall material or pretreated cell wall material is given.

*Measured after 30 min incubation. Phenoloxidase activity in absence of activating cell wall glucans was equivalent to A_{480} of 0.11 in the assay system.

†Figures in parentheses are A_{480} values in assay.

‡After 30 min pretreatment, 1,000% increase. After 3–5 d, increase was about 600%.

Thin-layer chromatography of the enzyme digests on silica gel (*n*-butanol/ethanol/water: 5/3/2) showed complete degradation of the glucan into mono- and disaccharides after 3 h of incubation with the enzyme.

Cell walls from many other fungi also activate the phenoloxidase from crayfish⁶. We treated activator-containing extracts from cell walls of *Polyporus annosus*, a basidiomycete, and *Aphanomyces astaci*, an oomycete, with the exo-glucanase as above. In both cases the activating capacity was destroyed indicating that the elicitor is a β -1,3-glucan and may be a general characteristic of most fungi.

Among vertebrate parasites fungi are less common than bacteria. The reason for this is not known and the vertebrate immune system cannot fully explain the discrepancy⁹. It is possible that soluble and chemically related cell wall fragments from different fungi, such as the elicitors described here, are also involved in the nonspecific recognition of fungi by vertebrates. It is interesting that a fungal β -1,3-glucan has been claimed specifically to induce anti-tumour activity in the mouse¹⁰.

Albersheim *et al.*^{11,12} have characterised fungal cell wall fragments which elicit host response in bean tissues, and the similarity with the fungal elicitor active in crayfish is striking. It is therefore tempting to speculate that most fungi cannot avoid producing 'warning signals' which are similar in different fungal groups and recognised by both plants and animals.

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TORGNY UNESTAM
KENNETH SÖDERHÄLL

*Institute of Physiological Botany,
University of Uppsala,
Uppsala, Sweden*

Received 22 November 1976; accepted 2 March 1977.

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Action of juvenile hormone mimics on the regulation of larval–adult and alary polymorphism in aphids

It has often been suggested that the development of the apterous and alate morphs of aphids is controlled by the corpus allatum (CA) and is therefore regulated by the same endocrine system that controls larval–adult development in monomorphic insects^{1–6}. Research on other polymorphic species, including the honey bee and termites, has shown that this function is compatible with the cyclical production of juvenile hormone (JH) during larval ecdyses as the morph-determining action of the hormone may be confined to a certain strictly defined critical period or may depend on specific hormone concentrations in the blood^{7,8}. In aphids the prenatal determination of late embryos as presumptive apterae has been attributed to high JH titres in the maternal haemolymph^{4–6}, and the subsequent realisation of the morph characters has also been thought to

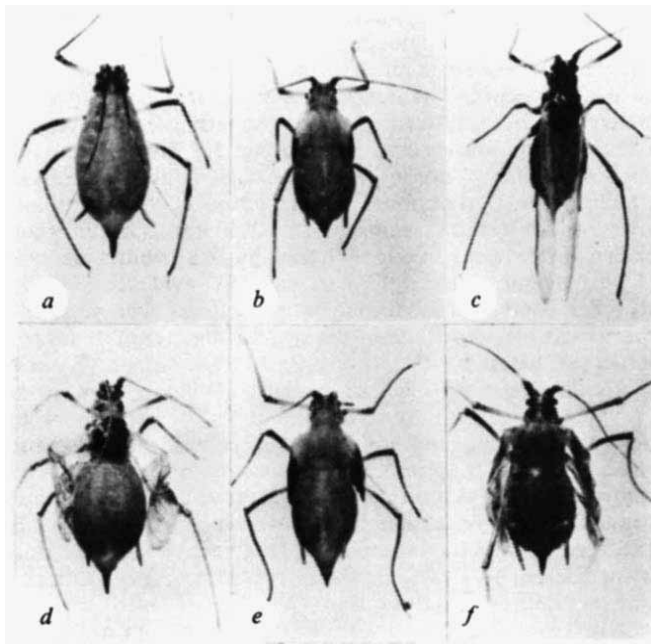


Fig. 1 *Megoura viciae*: a, normal adult aptera; b, normal alata in fourth (last) larval instar; c, normal adult alata; d, fifth-instar larval-adult intermediate produced by topical application of 10^{-3} μ g ZR-512 to a third-instar alate larva. This individual did not undergo a further ecdysis. e, Fifth-instar supernumerary alate larva showing maximum juvenilising effect. 10^{-2} μ g ZR-512 was applied to the third-instar alata. f, Sixth-instar larval-adult intermediate resulting from the ecdysis of a high grade supernumerary larva (as in e), produced by treating a third-instar alata with 10^{-2} μ g ZR-512.

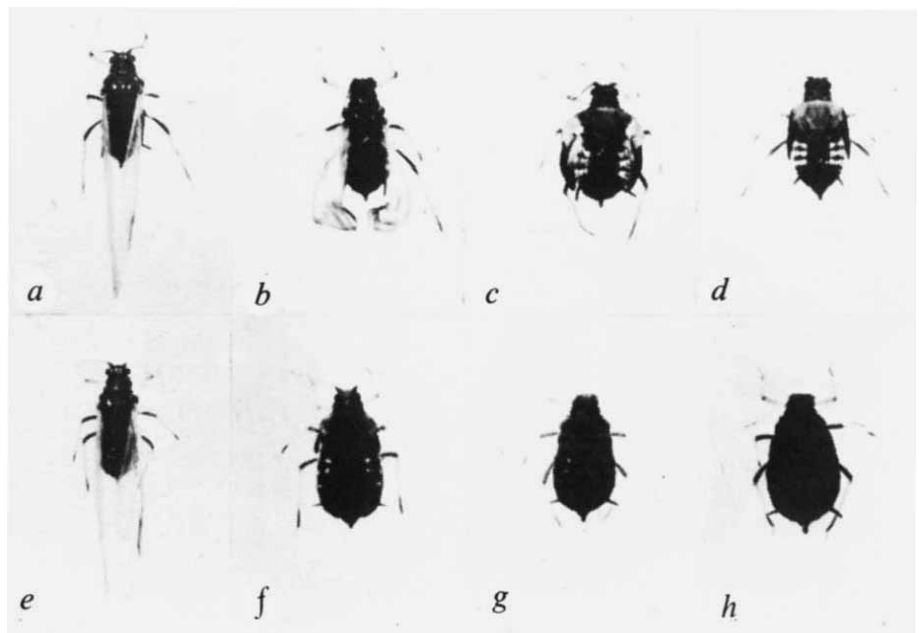
involve enhanced CA activity in the early instars of the developing larva^{2,3}. The evidence, which has been based on measurements of CA volume and on the effects of topically applied JH is nevertheless conflicting^{9,10}. The results reported here show that the intermediate and terminal forms produced by the application of juvenoids to developing alate aphids are unlike those resulting from authentic

apterisation initiated either by the absence of crowding or by exposure to long photoperiods. This does not support the persuasive argument that the possession of certain apparently juvenile characters by the 'neotenic' apterae (for example, the wingless condition of the adult) is a reliable indicator of JH involvement.

In early experiments with the vetch and bean aphid *Megoura viciae*, cecropia extracts with relatively slight JH activity were applied topically to alatform larvae in their penultimate (third) larval instar^{2,3}. After an externally normal ecdysis to the fourth instar, these individuals developed into adults with stunted wings, a reduced pterothorax and an abdominal pigment pattern resembling the adult aptera rather than the adult alata (Fig. 1a, c and d). The use of more potent synthetic JH mimics, however, has shown that this effect does not represent progress towards the apterous morph. After treatment with a saturating dose of JH mimic third instars develop into a supernumerary fifth-instar alate larva with unmistakable wing pads and a completely unsclerotised pterothorax (Fig. 1e). In addition, the genital opening remains closed and the ocelli are often suppressed. A further ecdysis follows. The few sixth-instar aphids that can extricate themselves from the old cuticle are often still somewhat juvenile in appearance (Fig. 1f). These more extreme features, which have been observed in several species^{5,11}, indicate that the juvenilising effect is similar to that in many other insects¹². It is not surprising therefore that other aphid morphs including the aptera and the wingless ovipara respond in a comparable manner^{13,14}.

It is less certain, however, whether juvenoids also affect morph determination, because this event occurs much earlier. In *Megoura*, for example, the morph type is controlled maternally just before birth¹⁵. To test this point, 1.0- μ g quantities of the juvenoids ethyl 3,7,11-trimethyl-dodecadienoate (ZR-512) (Zoecon), methyl 10,11-epoxy-farnesoate (C16-JH) (Eco-Control) or the Law-Williams (L-W) chlorinated farnesoate mixture¹⁶ were applied topically to the abdomen of parent apterae prepared as alata-producers by crowding them together¹⁷. Although all these growth regulators produce a maximum morphological response in the third-instar alata at a dose of only 10^{-2} – 10^{-3} μ g, the treated adults continued to produce alate

Fig. 2 *Aphis fabae*: a-d, range of forms produced by topical application of a JH mimic (Law-Williams) to second and third-instar gynoparae. Examples of four of the five arbitrary response categories are illustrated. a, Normal adult gynopara, untreated: 0% morphological effect (ME). b, Wings curled: 25% ME. c, Wings intermediate, pterothorax weakly sclerotised, large wax patches present: 75% ME. d, Maximal effect. A fifth-instar supernumerary larva with large larval wing pads and conspicuous wax patches: 100% ME. e-h, Range of forms produced by exposing presumptive gynoparae reared in short days to two long photoperiods during the first 2 d of development. The five response categories differ from the above. Examples of four are shown. e, Normal gynopara: 0% ME. f, Intermediate adult with small wing pads: 50% ME. g, Intermediate adult with some thoracic sclerotisation but no wing rudiments: 75% ME. h, Adult showing complete apterisation: 100% ME. Aphids placed in the 50% ME category in the JH series (not shown) have crumpled wings, partially obliterated sutures between the thoracic sclerites and small wax patches. Those assessed at 25% ME in the long day series have half-expanded wings but are otherwise normal alates.



progeny when subsequently isolated on bean seedlings (*Vicia faba*). Aptera-producers, isolated before the final moult, were also unaffected by JH treatments and yielded only apterae. In another experiment, the 1-d-old progeny of crowded parents were sprayed with an acetone solution containing 0.5 µg of L-W mixture (sufficient to ensure a maximum response in groups of third-instar alateform larvae). The proportion of insects developing as apterae was not increased. There was therefore no indication that these JH mimics either influenced the morph-determining process in the parent or modified the alate character of the neonatal progeny immediately after determination.

The winged gynoparae of a host-alternating species such as the black bean aphid *Aphis fabae* have two advantages as experimental subjects. First, it is certain that every first-instar larva is a presumptive alata, provided the parent has developed in short days (12 h light: 12 h dark) at 15 °C. Second, the alate condition of the first-instar gynoparae is partially or completely suppressed by exposure to a threshold number of long days (16 h light: 8 h dark) during the first and second instars. The intermorphs and extreme forms can then be compared with the intermediates produced by JH treatments.

The range of morphological effects produced by JH is shown in Fig. 2. As in *Megoura*, the extreme forms are near-perfect fifth-instar supernumerary alate larvae with large wing pads, an unsclerotised pterothorax, a closed genital canal and, in this species, conspicuous larval wax patches (Fig. 2d). These juveniles invariably undergo an additional ecdysis. An assay based on these and other characters has been used in assessing the morphological effect (ME) of a JH mimic (0.5 µg L-W in acetone) when applied as an atomised spray to developing colonies of gynoparae, each comprising about 100 individuals. Figure 3 shows that the presumptive gynoparae display relatively little sensitivity in the first day or two of post-natal development, the single application of mimic again producing its greatest effect in the third instar (day 7). In contrast, exposure to two consecutive long photoperiods is maximally effective during the first 2 d of development (Fig. 3). As a result of this early treatment 67% of the presumptive gynoparae continued to develop as normal alate morphs (Fig. 2e), 8% developed as intermediates, some with small wing pads (Fig. 2f), while 25% developed as authentic apterae

without wing vestige or thoracic sclerotisation (Fig. 2h). Moreover, the intermediates and apterae gave birth to parthenogenetic daughters (virginoparae), whereas the unchanged alatae produced oviparous progeny as would a normal gynopara. Neither the intermediates nor the completely apterised aphids underwent supernumerary ecdyses. These results show that juvenilising JH treatments and photoperiodically controlled apterisation are maximally effective at different times during larval development and give rise to terminal morphs which differ both in their external form and in such physiological attributes as type of germarium and ecdysis pattern. This evidence suggests that the control of wing polymorphism, whether vested in the parent (*Megoura*) or in the developing larva (*A. fabae*), is not mediated by JH.

Aphids that have been determined as alata-producers by crowding give birth to a small number of progeny when decapitated^{18,3}. These are invariably apterous. The same effect can also be produced in *Megoura* by sectioning the central nervous system at the level of the suboesophageal ganglion and by other treatments which would not be expected to affect CA activity. These include the exposure of the parent to CO₂ anaesthesia for 30 s, and the amputation of the distal extremities of the legs. The latter effect, which is not produced by other comparable injuries, such as antennal amputation, seems to be correlated with the removal of certain whip-like trichoid sensilla adjacent to the eversible tibial climbing organ. Evidently, the loss of the normal sensory input to the thoracic ganglion both releases the parturition reflex from inhibition and elicits the morph change in the fully developed embryo. These considerations suggest that the effector is neural, but there is as yet no evidence that the control mechanism involves a blood-borne hormone.

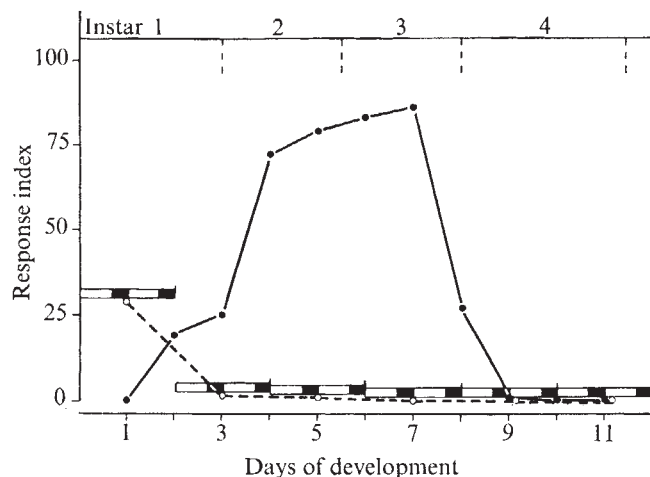
A. D. LEES

ARC Insect Physiology Group,
Imperial College Field Station,
Silwood Park, Ascot, Berkshire, UK

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Fig. 3 Time of responsiveness to treatments influencing juvenile-adult and apterous-alate characters in gynoparae of *Aphis fabae*. —, Response to a single application of L-W juvenoid when applied at constant dose on different days of larval development. — — —, Response to two long days applied on the days indicated by the photoperiod bars. The response index is calculated as $\Sigma (ME \times n)/N$, where ME is the percentage morphological effect, n the number showing this grade of response and N the total number of individuals.



Growth and differentiation of a primitive nervous cell line after *in vivo* transplantation into syngeneic mice

In vitro study of nerve cell differentiation has been primarily concentrated on the examination of cell lines that, at least partially, express terminal steps of differentiation. The isolation and characterisation of precursor populations would be an aid to further understanding of nerve cell differentiation. I present here evidence for a clonal line of such a nerve cell precursor type which, after *in vivo* passage, is able to give distinct neuronal phenotypes. These phenotypes are potentially lineage related, and show qualitative

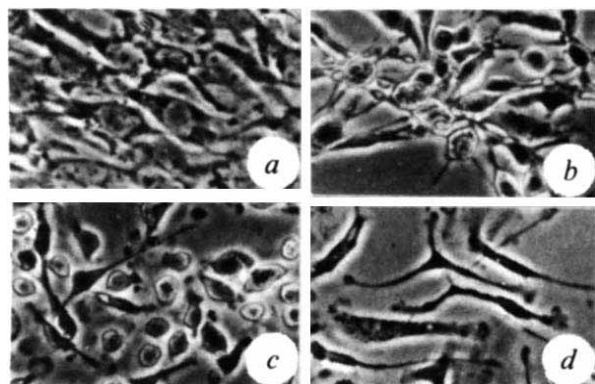


Fig. 1 Diversity of tumour clones. Phase contrast photographs of four hypothalamic clones issued from a tumour of the primitive F7 cells. *a*, A primitive tumour clone. *b*, A newly differentiated cell type (4-3). *c* and *d*, Two neurosecretory cell types as compared to C7 clone's morphology¹. Tumour was sterily excised, finely minced with scissors and then mechanically disrupted with a Pasteur pipette. Cells and small aggregates were plated in 30 ml Falcon plastic culture bottles containing 2-3 ml of Ham F10 medium GIBCO supplemented with 15% heat inactivated horse serum, 2.5% foetal calf serum, 1.5% of 200 mM L glutamine, penicillin (50 IU ml⁻¹) and streptomycin (50 µg ml⁻¹). Cells were grown in an atmosphere of 10% CO₂ 90% air.

differences in the expression of their hypothalamic neuronal function.

Clonal cell line C7 isolated from primary cultures of 14-d-old mouse hypothalamus embryo (A/J) after SV 40 transformation initially retained the capacity to form neurophysin and vasopressin¹, a function unique to some hypothalamic neurones *in vivo*. After several passages however, some cells lost the capacity to synthesise neurophysin and vasopressin. Subclones of the original C7 line were isolated and morphologically, histochemically and immunologically characterised. The primitive nature of one of these clones, F7, derived from such neurosecretory cells, has been tentatively established on the basis of its ultrastructural features and its negative response to all of the tests that were used to identify neurosecretory cells².

If it were possible to demonstrate that a reversible switch from this primitive phenotype F7 back to the differentiated one had occurred, it would give a means of determining whether F7 is a precursor cell antecedent to a definitive neurone; although such a reversible switch has not been observed *in vitro*, this differentiation could be initiated by passage of these primitive nerves through syngeneic mice. The proliferating cells originating from these primitive nerve cells, after transplantation in mice, give rise to tumour cell populations containing both primitive and differentiated cells, as defined by the presence of immunoreactive neurophysin and β -glucuronidase activity.

The primitive nerve cell clone F7 (10⁷ cells) was injected subcutaneously into male A/J mice. Tumours arose after a latent period of 5-10 d. The ability of such transformed cells to proliferate was surprising in view of the fact that the original C7 neuronal type was unable to produce tumours in the same conditions. These tumours were excised and cultivated. Primary cultures were maintained for about 6 weeks in the same flask before cloning and medium was changed by half every 2-4 d. Cells originally plated grew into three distinct morphological types (Fig. 1*a-d*) among which one type was predominant (type 1*b* in Fig. 1). Colonies of these three distinct phenotypes were dissected out with a 10 µl Pedersen micropipette, scraping only the centre of the colony to get clean cell populations.

The first resulting homogeneous population of cells grew in a non-overlapping monolayer, with a division time of 12-16 h (Fig. 1*a*). The model chromosome number of the cells was the same as for the original F7 clone (70

chromosomes). This population grew as a stable line. The Gomori's paraldehyde fuchsin and β -glucuronidase histochemical reactions used before to identify the neurosecretory cells were consistently negative on these primitive cells². The immunocytochemical detection of neurophysin using bovine neurophysin I and II antisera also gave negative results. These cells closely resembled the originally inoculated F7 cells.

The second class of cells were very refractile and interconnected by long narrow processes; they had a division time of about 24 h (Fig. 1*b*). These cells were positive for the two histochemical markers of neurosecretory cells: the Gomori's paraldehyde fuchsin and β -glucuronidase reactions (Table 1). They gave a positive immunochemical reaction for neurophysin using bovine neurophysin I antiserum (Fig. 2). They gave a negative reaction using bovine

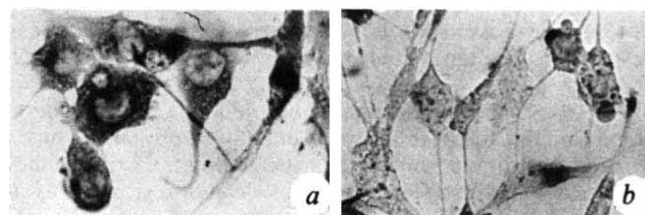


Fig. 2 Immunochemical staining with Ab-NI (1/50). *a*, A newly differentiated tumour cell clone (4-3). *b*, A primitive tumour cell clone (PT). ($\times 300$.)

neurophysin II and vasopressin antisera, however (Table 1). These cells were unstable in the culture conditions—4 to 5 months after the excision of the tumour corresponding to about 17-20 subcultures, the level of intracellular neurophysin began to decline as indicated by the immunochemical detection. At the same time, these cells started to divide faster, and progressively took the phenotype of the originally fast dividing inoculated F7 cells.

A third type of cell could be identified among the heterogeneous population derived from the tumour: a neuronal refractile cell type (Fig. 1*c* and *d*) closely resembling the

Table 1 Results of the cytochemical and immunocytochemical staining of the primitive tumour cell type and the newly differentiated tumour cell type, as compared to the original clones (C7 and F7)

Clone	Gomori's paraldehyde fuchsin	β -glucur- onidase	Ab N I 1 : 50	Ab N II 1 : 10	Ab LVP 1 : 10
Tumour Primitive type (PT)	—	—	—	—	—
Differentiated type (4-3)	++	++	++	—	—
Control neuro- secretory clone (C7)	+++	+++	+++	+++	+++
Primitive clone (F7)	—	—	—	—	—

Gomori's paraldehyde fuchsin staining and cytochemical detection of β -glucuronidase were as previously described². Neurophysin and vasopressin were localised using the peroxidase-labelled antibody technique with antibodies against bovine neurophysin II (Ab-N II), bovine neurophysin I (Ab-N I) and Lys-vasopressin (Ab-LVP). Cells grown in plastic dishes were treated according to Tougaard *et al.*⁷. The specificity of the reaction was tested by using normal rabbit serum. Rabbit antiserum to bovine neurophysin II was specific for neurophysin II and cross-reacted very poorly with bovine neurophysin I⁸. A dilution of 1/10 was used. Rabbit antiserum to bovine neurophysin I cross-reacted slightly with N II (ref. 9). A 1/50 dilution was used. Anti-lys-vasopressin serum was obtained in rabbits immunised with vasopressin coupled to charcoal. A 1/10 dilution was used.

neurophysin-vasopressin synthesising C7 clone¹. These cells grew in a dispersed state, and the number of colonies of such type was small as compared to the other types. Their division time was about 2–3 d. They were rapidly overgrown by other much faster dividing type of cells and they could not be dissected or subcultivated. On the basis of their morphological analogy with the other neurophysin-vasopressin clones already isolated², and their slow growth rate as compared to the fast growth rate of the F7 type clones, they may represent the neurophysin-vasopressin synthesising cell class.

In summary the primitive nerve cell line F7, when injected subcutaneously into syngeneic mice, gives rise to tumours which after subculture contain not only primitive cells but also a new type of differentiated cells. These newly differentiated cells can be distinguished from primitive cells on the basis of their morphological and growth rate alterations, their neurophysin content, and their positive reaction towards β -glucuronidase activity, a neurosecretory cell marker *in vitro*². These cells which contain neurophysin but are negative with a vasopressin antiserum may correspond to a step of differentiation towards the neurosecretory type.

These observations support the view that cells undergo stable modifications in their differentiated properties in the *in vitro* conditions, but retain the ability to express their specific function under favourable conditions such as *in vivo*. It seems unlikely that the changes observed in the populations could be due to selective growth of pre-existing neurophysin cell type, since the F7 cell population inoculated into the animal originated from a single cell progeny, and grows as a stable line. Its growth rate being about four times faster than the neurophysin-vasopressin synthesising cell type, it has a selective advantage in the culture conditions, and would rapidly overgrow a neuronal cell, if any. If F7 cells were able to segregate neurophysin synthesising cells *in vitro*—it could not be observed with our markers—this would argue for F7 being a precursor cell type of a neurophysin cell. Finally inoculation of 10^7 cells of the neurophysin-vasopressin synthesising C7 clone¹ into syngeneic mice did not give any tumour.

The *de novo* synthesis of a differentiation gene product associated with the *in vivo* passage is a striking phenomenon. Such induced morphological and cell function alterations have been reported in the case of endocrine tumour cells: hormonal activity may be restored in tumour cells which no longer synthesise their specific product, by animal passage³. In the case of teratocarcinoma, lines can be established that remain undifferentiated. Some of these, when injected into mice, give rise to tumours containing various tissues^{4–6}. In our case more studies are needed to determine whether this primitive cell F7 is only a precursor of a neurosecretory cell lineage or is a more remote stem cell for nerve cell types such as glial cells or other types of neurones. But these cells might be used as a suitable model to study the sequence of steps involved in the progressive differentiation of a neuronal lineage.

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F. DE VITRY

Groupe de Neuroendocrinologie Cellulaire,
Laboratoire de Physiologie Cellulaire,
Collège de France, 11 Place Marcelin Berthelot,
75231 Paris 05, France

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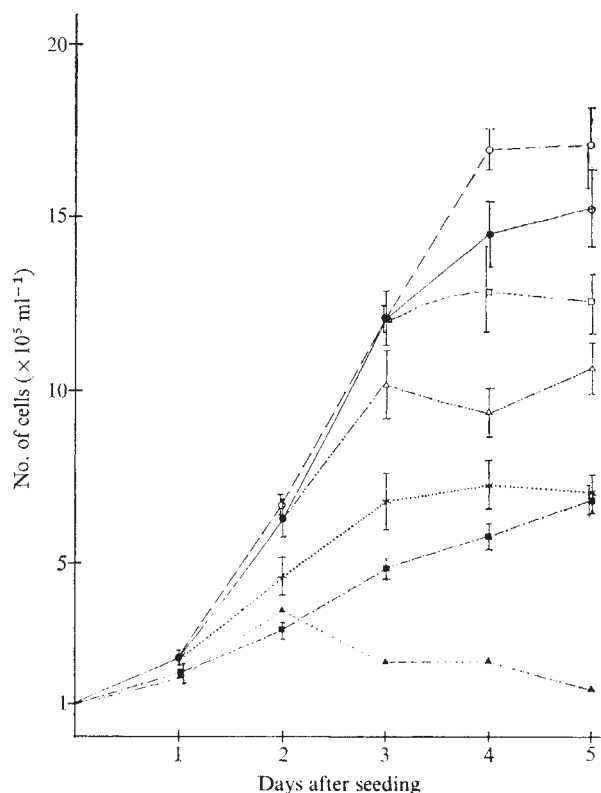
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Interferon inhibits dimethyl sulphoxide-induced erythroid differentiation of Friend leukaemia cells

INTERFERON (IF), as well as showing antiviral activity is able to inhibit cell growth and metabolism both *in vivo*^{1,2} and *in vitro*^{1,3–5}. In the *in vitro* systems used, dead cell counts are always very low, indicating that IF inhibits cell growth by decreasing their doubling potential^{4,5} without appreciable cell death. Since we have shown previously that IF inhibits *in vivo* growth of Friend virus (FLV)-induced murine erythroleukaemic cells (FLC)⁶, we have studied the *in vitro* effects of IF addition to cultures of FLC. These cultures, on exposure to dimethyl sulphoxide (DMSO), undergo massive erythroid differentiation accompanied by haemoglobin (Hb) synthesis^{7,8} as a consequence of production of globin messenger RNA^{9,10}. Here we present evidence for a dose-dependent and reversible inhibitory effect of IF on both growth and erythroid differentiation of DMSO-stimulated FLC.

Several preparations of interferon were used in the

Fig. 1 Growth curves of Friend leukaemia cells (FLC) treated with DMSO and/or IF. FLC (clone 745A from C. Friend), grown in suspension cultures in Dulbecco's modified Eagle's medium, supplemented with 15% foetal calf serum and antibiotics, kept at 37 °C, were seeded at 10^5 cells per ml with no further treatment (○); with 1.5% (by vol.) DMSO (●) and with 1,000 units of IF (■). To samples of 1.5% DMSO-treated cultures, 1,000 U of IF were added either on day zero (▲), or on day 1 (×), or on day 2 (△), or on day 3 (□). Data shown are means $\pm$ s.e.m. of viable (trypan blue dye-exclusion method) cell counts from several experiments. Mouse IF preparations were from Drs I. Gresser and F. Dianzani. Procedures for IF production, partial purification and titration have been described¹⁶.



present study: IF dosages are given in mouse interferon units equal to 4 mouse interferon reference standard units.

IF was initially added at the time of cell seeding (day zero) without DMSO. A pilot experiment showed that IF at 1,000 units per ml per 10^5 cells inhibited FLC growth five-fold with no cell death, as judged by trypan-blue dye exclusion method (Fig. 1, ■). This inhibition was markedly enhanced when IF and DMSO were given simultaneously on day zero. In these conditions, where not even one round of cell doubling occurred and a few dead cells were observed on days 4 and 5, no Hb synthesis was detectable. This is in keeping with reports^{11,12} that at least one cell doubling is required for DMSO induction of Hb synthesis, although this is not necessarily true for other inducers such as butyric acid¹³.

When, instead, IF was added 24 h later (day one) to cultures seeded with DMSO so that the cell population was allowed to undergo at least one doubling before being exposed to IF (Fig. 1, ×), the addition of IF still inhibited cell growth. The IF-treated cultures reached on day 5 a concentration of about 7×10^5 per ml without appreciable mortality. This implies that the cells doubled almost twice after the addition of IF. In these conditions, used in most experiments described hereafter, a ninefold reduction of Hb production, as measured on the fifth day of culture, was observed (Table 1). Hb values of DMSO+IF-treated FLC remained low even when tested on day 7. This effect was much less evident when IF was added on day 2 or 3. On the other hand, there was only a twofold reduction in the accumulation of ⁵⁹Fe and its incorporation into haem.

The IF effects on cell replication and differentiation are dose-dependent (Fig. 2). It is noteworthy that at least 500 units of IF per ml per 10^5 cells are needed to appreciably detect them. This most probably accounts for the apparent discrepancy between our data and those of Swetly and Ostertag¹⁴ and Lieberman *et al.*¹⁵, who found that doses of IF capable of inhibiting FLV production by FLC *in vitro* had no appreciable effect on cell growth and DMSO-induced erythroid differentiation of these cells.

That the observed effects are attributable to IF itself rather than to unknown contaminants of IF preparations is indicated by the following evidence. First, effects induced by IF preparations of various degrees of purification ($\geq 10^6$ units per mg protein for Gresser preparations, 10^5 or 10^4 units per mg protein for Dianzani preparations) are indistinguishable. Second, mouse IF proved equally active regardless of the cell type used as source of IF (brain, fibroblasts, ascites cells) and of the various inducers (West Nile virus or Newcastle disease virus). And third, rabbit heterologous IF or mouse mock IF (medium from uninduced cultures) were ineffective (data not shown).

Inhibition of growth and differentiation of FLC can be fully reversed by thorough washing to remove IF (Table 2). Growth curves of IF-treated cell cultures, following washing

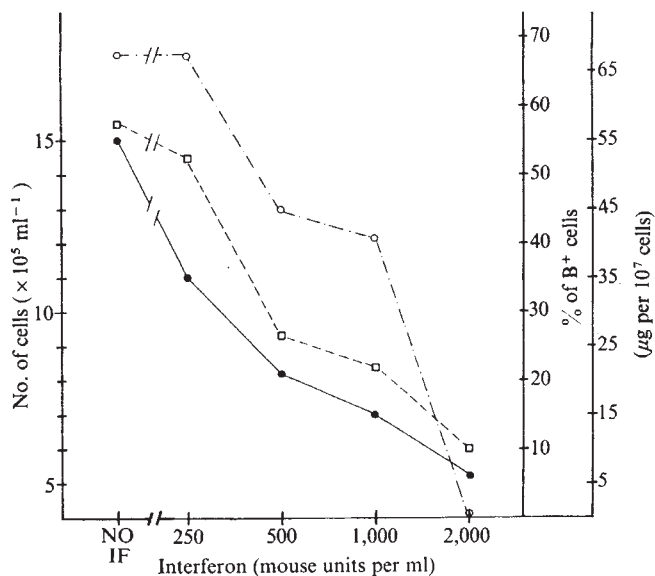


Fig. 2 Dose response curves of IF inhibitory effect on FLC growth and DMSO-induced differentiation. Data are the average of triplicate samples of 5-d cultures seeded with 1.5% DMSO and given the indicated IF amounts on day 1. Benzidine-positive (B+) cells counted on cyto-centrifuge sediments stained with Ralph's technique. At least 500 cells were scored. Viable cell counts as for Fig. 1. Haemoglobin measurements were carried out on cell lysates as described for Table 1. ●, No. of cells per ml. □, % B+ cells. ○, $\mu\text{g Hb per } 10^7 \text{ cells}$.

and reseeded at 10^5 cells per ml are indistinguishable from controls, when checked daily. Similarly, reversal of the inhibition of erythroid differentiation has been accomplished. As shown in Table 2, when cells induced with DMSO and treated with IF under the usual conditions were washed and reseeded at high and low density in fresh DMSO- and IF-free medium, amounts of Hb detected in cells that had or had not been treated with IF were comparable. When, however, cells obtained at the fifth day of culture after DMSO+IF treatment, were washed and reseeded into DMSO-supplemented medium, they were fully reinducible by DMSO indicating that IF treatment had not merely selected a DMSO-resistant variant.

Thus our results suggest a marked inhibitory capacity of IF on DMSO-induced Hb production by FLC. It should be emphasised that the decrease of Hb synthesis occurs in conditions where the overall protein synthesis is not affected by IF treatment. This was determined by measuring acid-precipitable radioactivity after pulses or continuous labelling with ³H-arginine (data not shown). The IF-induced inhibition of Hb synthesis is reversed by removal of IF, further

Table 1 Effect of IF on ⁵⁹Fe accumulation, its incorporation into haem and Hb production of FLC cultures

Treatment	⁵⁹ Fe accumulated (c.p.m. per 10^7 cells)	⁵⁹ Fe incorporated into haem (c.p.m. per 10^7 cells)	Hb ($\mu\text{g per } 10^7 \text{ cells}$)
None	13,251	1,421	4.76
DMSO 1.5%	38,402	3,632	47.46
DMSO + IF on day 1	19,922	1,872	5.52
DMSO + IF on day 2	36,435	4,505	29.30
DMSO + IF on day 3	39,649	4,389	25.24

Data shown are for samples obtained at day 5 for culture from a representative experiment. FLC cells were seeded at the density of 10^5 per ml with or without 1.5% DMSO in presence of $0.1 \mu\text{Ci ml}^{-1}$ ⁵⁹Fe-citrate. 1,000 units of IF were added to separate culture samples at time intervals indicated. On day 5, cells were washed, resuspended in 3.0 ml distilled water, and lysed by repeated freeze-thawings. Aliquots of lysates were counted for radioactivity (⁵⁹Fe accumulated). Haem was extracted from lysates according to Scher *et al.*¹⁷ and the radioactivity of such extracts counted (⁵⁹Fe incorporated). Haemoglobin was measured from cell lysates according to Crosby and Furth¹⁸.

Table 2 Reversal of IF inhibition of growth and differentiation of DMSO-stimulated FLC

Treatments	DMSO-treated FLC		DMSO + IF-treated FLC	
	No. of cells per ml ($\times 10^{-6}$)	Haemoglobin ($\mu\text{g per } 10^6 \text{ cells}$)	No. of cells per ml ($\times 10^{-6}$)	Haemoglobin ($\mu\text{g per } 10^6 \text{ cells}$)
5 days of culture	18.3	55.4	5.5	7.2
Cells from above conditions were then treated thus:				
a, Washed and reseeded in DMSO- and IF-free medium at 10^5 cells per ml*	9.2	36.5	9.7	22.7
b, Washed and reseeded in DMSO- and IF-free medium at 10^6 cells per ml*	15.1	37.2	14.8	37.9
c, Washed and reseeded in DMSO-supplemented IF-free medium at 10^5 cells per ml†	15.0	36.4	14.7	42.5

Culture conditions as for Fig. 1. 1,000 Units of IF added on day 1. Haemoglobin measured according to ref. 18.

*Tested 48 h after treatment.

†Tested 96 h after treatment.

confirming the specific character of its effect. The prompt resumption of Hb production in absence of DMSO after IF removal suggests that IF action had not effaced the DMSO imprinting of FLC.

Work is in progress to determine, at the molecular level, the mechanism by which IF addition inhibits Hb synthesis. It is apparent, however, that globin synthesis or its assembly into Hb rather than haem production is the most likely target of IF action.

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G. B. ROSSI
G. P. MATARESE
C. GRAPPELLI
F. BELARDELLI

Section of Virology,
Istituto Superiore di Sanità,
299 Viale R. Elena,
00161 Rome

A. BENEDETTO

Center of Virology, Ospedali Riuniti,
87 Circonv. Gianicolense,
00152 Rome, Italy

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Polyclonal Ig production after Epstein-Barr virus infection of human lymphocytes *in vitro*

APART from the specific anti-Epstein-Barr virus (EBV) response in infectious mononucleosis (IM), there is a strong general increase of antibody levels¹. The major increase comprises IgM. As EBV has been shown to infect B lymphocytes selectively² we hypothesised that the virus triggered the infected cells to release immunoglobulin, much like the effect of certain B-cell mitogens³, although the mechanism of activation must be different. It is known that EBV infection *in vitro* of human blood lymphocytes gives rise to increased DNA synthesis⁴ and that lymphoid cell lines carrying the EBV genome shed or release immunoglobulins⁵ of various classes. Furthermore, the IgM increase seen in IM would fit with a polyclonal B-cell activation (PBA) of EBV as PBA usually gives rise to IgM secretion⁶. To test this hypothesis, we have exposed lymphocytes from cord blood and adult peripheral blood to EBV *in vitro* and measured the released IgM, by a double-antibody radioimmunoassay, and individual antibody-forming cells (PFC) against haptenated red blood cells and sheep red blood cells (SRBC).

Lymphocytes, both from EBV seronegative and EBV seropositive donors were infected with strain B95-8 of EBV. Table 1 shows that DNA synthesis, as measured by ³H-thymidine incorporation, increased considerably over the non-infected control cultures, which, however, were stimulated to some degree by the foetal calf serum (FCS) in the medium. The stimulation ratios between infected and control cultures varied from 1.5 to 20 and there was no obvious difference between lymphocytes from cord blood and adults. The amplitude of activation seemed to be specific to each donor, with no correlation with the origin of the lymphocytes. We wished to know whether the B lymphocytes were triggered to become Ig-producing cells, because these infections mimic the *in vivo* situation found in IM. IgM was present from day 3 after infection, as measured by radioimmunoassay, and increased in most cultures by a factor of 10 until day 7 (Table 1). Lymphocytes from adult EBV positive donors secreted detectable IgM in control cultures (Table 1) but at a significantly lower level than in virus-stimulated cultures. The double-antibody radioimmunoassay for IgM was specific for human IgM, and no interference with other human proteins or FCS proteins was observed.

To confirm the radioimmunoassay results, we collected spent media from infected and control cultures and labelled them with ¹²⁵I. Monospecific antibodies against μ , γ , κ and λ chains were used to precipitate out Ig from the bulk of other proteins in the spent medium. The results indicate (Table 2) that the Ig produced is polyclonal, because all

Table 1 IgM production of EBV-infected human lymphocytes after 7 d

Lymphocyte source	(ng IgM per 10 ⁶ cells)*	³ H-thymidine incorporation (c.p.m.)	EBNA induction (% positive cells) †
G.L. adult blood EBV positive	360/30	48520/9056	0.5
G.Li. adult blood EBV positive	290/7	32105/1602	21
N. A-G. adult blood EBV positive	30/0	ND/1225	1.5
K.H. adult blood EBV negative	460/ND	ND/1510	8
I.E. adult blood EBV negative	115/30	1910/ND	ND
J.E. adult blood EBV positive	245/ND	6147/946	4
B.A. adult blood EBV positive	250/125	ND	ND
A.P. adult blood EBV positive	54/8	8112/1952	5
K.G. adult blood EBV positive	160/84	128442/8267	17
N.C. IM-blood EBV positive	9/0	9612/812	0.5
P.H. cord blood EBV negative	10/0	25878/5983	9
A.Z. cord blood EBV negative	960/0	68741/7357	12
E.K. cord blood EBV negative	ND	140878/19827	14

Figures in columns 2 and 3 are infected over control.

*Sensitivity of radioimmunoassay: 3 ng ml⁻¹. ND, not done.

†Values from infected cultures; control cultures were always negative.

Lymphocytes were separated on Ficoll-Isopaque¹⁷. Two batches of EBV (supernatant of B 95-8 cells (ref. 7) filtered through a Millipore membrane pore size 0.45 µm) were used. Lymphocytes were pelleted and resuspended in B 95-8 supernatant (1 ml was added to 10⁷ cells). The cells were incubated for 1 h at 37 °C with continuous shaking. Thereafter the cells were washed three times with medium (RPMI 1640, supplemented with 10% FCS and antibiotics), finally they were resuspended in medium to a concentration of 10⁶ cells per ml. Cells were incubated for 7 d at 37 °C in 5% CO₂ and 95% air in 50-ml plastic bottles (Falcon plastics). 16 h before collection 10⁶ cells were transferred into Falcon microplate wells (2 × 10⁵ cells in each well) and 1 µCi ³H-thymidine (Amersham, specific activity: 5 Ci mmol⁻¹) was added to each well. The cultures were sucked off with the aid of multiple sample processor (Skatron, Norway) to glass fibre filters. After drying, the filters were placed in vials containing scintillation fluid. The isotope determinations were made by a Packard Tri-Carb scintillation spectrometer and expressed as c.p.m. EBV-determined nuclear antigen (EBNA) staining was carried out on day 7 according to the method of Reedman and Klein¹⁸. A double-antibody radioimmunoassay was established for measurements of minute amounts of IgM. IgM was isolated from a pool of human serum by precipitation with 50% saturated ammonium sulphate, washed three times with 33% saturated ammonium sulphate, dialysed against 0.1 M Tris-HCl buffer, pH 7.4, containing 1 M NaCl and fractionated on Sephadex G-200 in the same buffer. The first peak was pooled and fractionated by Pevecon block electrophoresis in Veronal-HCl buffer, pH 8.6, *I* = 0.1. Isolated IgM was kept in a 0.1% glycine buffer in phosphate buffered saline, pH 7.4, to avoid aggregation. The purified IgM was labelled with ¹²⁵I according to a modification to Hunter and Greenwood¹⁹. Radioimmunoassay was performed as follows: 10 µl spent medium or standard (purified IgM) in twofold dilutions from 25 to 0.05 ng was incubated for 4 h, 37 °C with 10 µl of rabbit anti-IgM (Dakopatts, Copenhagen), diluted 1:4,000 plus 25 µl of normal rabbit serum diluted 1:500. Then 10 µl of labelled purified IgM (0.5 ng labelled with 5,000–10,000 c.p.m.) added and the mixture incubated for 1.5 h at 37 °C. 25 µl of sheep anti-rabbit globulin 1:50, was then added for 18 h of incubation at 4 °C. All reagents were diluted in a 10 mM Tris-HCl buffer, pH 7.5, containing 1 mM EDTA, 15 mM NaCl and 0.4% Triton X-100 and 20 mg bovine serum albumin (BSA) per ml. Precipitates were washed three times in cold buffer without detergent and BSA and counted in an Intertechnique γ-counter. A standard curve was plotted and the concentration of IgM of the unknown samples from the spent medium of infected and non-infected cultures were determined according to the standard curve. A similar radioimmunoassay for Ig light chains has recently been described by Rosén *et al.*²⁰.

four antisera showed significant precipitation values. Auto-radiographic analysis of the radioactive precipitates on slab gels (4–25% gradient sodium dodecyl sulphate–polyacrylamide gel, in non-reducing conditions) confirmed two facts: 19S IgM was synthesised and both IgG and IgM were produced by the stimulated cells (unpublished results of A. R. and R. Laskov). The control culture precipitates showed weak bands in the positions of IgM and IgG but the quantitative differences were striking.

We have looked for several antibody specificities, including the heterophile antibody in the immunoglobulins from the supernatants of the EBV-stimulated cultures, but without success. However, when testing for single antibody forming cells (Table 3) in a modified plaque assay* after 6 d of culture, we found that EBV-exposed cord blood lymphocytes generated direct PFC against both haptenated RBC and SRBC. The frequencies were of the same order as with cells exposed to 20 µg of pokeweed mitogen. Single antibody-forming cells were developed following the principles described by Jerne and Nordin²¹, but essentially with the modifications for human effector cells described by Fauci and Pratt²². Purified blood lymphocytes (5 × 10⁶) were spun down and incubated in 1 ml of supernatant of B 95-8 for 60 min at 37 °C. After two washes the cells were incubated

in flasks (Falcon 3013) at 10⁶ per ml in RPMI in 10% FCS and 5% CO₂ in air. After 3–7 d the cells were collected and plated on thin-layer agarose as described by Bullock and Möller¹⁰. The target cells were SRBC or haptenated (FITC) SRBC (ref. 11). Only direct PFC were studied. Guinea pig serum diluted 1:40 was the source of complement. As a B-cell mitogen, 20 µg of pokeweed mitogen was added to 5 × 10⁶ lymphocytes for the whole culture period.

Between 50 and 90% inhibition of the number of plaques formed was observed when a rabbit anti-IgM antiserum was added before plaques were developed. This indicated that IgM antibodies caused plaque formation.

EBV nuclear antigen (EBNA) (ref. 18) was induced as a result of infection (Table 1). The appearance of EBNA agrees with the assumption that the stimulation of B cells was due to the penetration of EBV into the cells.

We have demonstrated that peripheral blood lymphocytes from cord blood and adults make immunoglobulins after exposure to EBV *in vitro*. The impact of this finding is threefold. First, we believe that it represents an *in vitro* correlate of the *in vivo* infection with EBV in IM¹² because there is an increase of polyclonal IgM in this disease¹. Our demonstration of IgM and IgG with κ and λ chains by immunoprecipitation and single PFC with at least two antibody specificities (anti-FITC and anti-heterologous RBC) suggests polyclonality of these antibodies induced *in vitro*. Ig secretion has been reported⁹, by established lymphoid lines made immortal by the addition of EBV¹³. This study, however, reveals the early events, before the establishment of a line.

Our data show that human newborn and adult peripheral blood contains cells that can be induced to produce antibodies. Antibody-producing potential is thus not confined to stationary cells of lymphoid organs, and age does not seem to influence this capacity at least with regard to IgM

Table 2 Precipitation of Ig from ¹²⁵I-labelled supernatants

Antibody	Donor 1	% Precipitated*	Donor 2
Anti-μ chain	1.0		1.0
Anti-γ chain	0.8		1.2
Anti-κ chain	0.3		0.3
Anti-λ chain	0.4		0.3

The precipitation systems were optimised by titrating purified IgM or IgG against 50 µl of the rabbit antisera.

*Infected minus control.

Table 3 Number of antibody-forming cells (PFC per 10^6 recovered cells) in human blood lymphocytes after *in vitro* exposure to EBV and pokeweed mitogen

Material added <i>in vitro</i>		EBV*		Pokeweed mitogen†		None	
Cell source		FITC-SRBC-PFC	SRBC-PFC	FITC-SRBC-PFC	SRBC-PFC	FITC-SRBC-PFC	SRBC-PFC
Cord blood	1	140	40	180	110	0	0
	2	210	65	235	160	15	0
	3	95	30	125	70	0	0
	4	180	45	140	100	10	0
Adult blood	1	165	40	280	165	25	0
	2	85	35	175	170	0	0
	3	240	155	270	235	15	15
	4	260	230	295	180	0	0

Plaque assay was done after 6 d culture, both against FITC-coupled sheep red blood cells (FITC-SRBC-PFC) and SRBC alone (SRBC-PFC).

*A 1-ml sample of EBV from B 95-8 supernatant was added to 5×10^6 in 2 ml of purified blood cells from umbilical cord and adult donors. of unknown immune status to EBV. The mixture was incubated for 1 h at 37 °C and the cells were washed and cultured.

†20 µg pokeweed mitogen was added at start of culture.

antibodies. Finally, we believe that EBV activation will be helpful in the study of B-cell triggering as the virus binds to B lymphocytes by means of receptors that are intimately associated with the complement receptors¹⁴. There is still controversy^{15,16} as to whether mere binding to the complement receptor will result in full activation of the B lymphocytes. We intend to explore this area, utilising the same experimental system, by exposing blood B lymphocytes to both non-transforming and heat inactivated EBV, respectively, both of which supposedly bind to B lymphocytes by means of the same receptor as normal EBV. We also plan to investigate whether the antibody-forming cell is EBNA positive in these conditions and, if so, whether an EBNA positive B cell can make anti-EBV antibodies.

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ANDERS ROSEN
PETER GERGELY
MIKAEL JONDAL
GEORGE KLEIN

Department of Tumour Biology,
Karolinska Institutet,
S-104 01 Stockholm, Sweden

SVEN BRITTON

Department of Immunobiology,
Karolinska Institutet,
Wallenberg Laboratory, Lilla Frescati,
S-104 05 Stockholm, Sweden

Received 11 February; accepted 9 March 1977.

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Prevention of oncornavirus-induced sarcomas in cats by treatment with antiviral antibodies

IN mice, infections with leukaemogenic viruses, as well as subsequent development of disease, can be permanently suppressed by treatment with antibodies specific for the major glycoprotein (gp71) of Friend murine leukaemia virus (MuLV Friend)^{1,2}. Similar protocols using antibodies

to feline leukaemia virus (FeLV) result in elimination of FeLV infection in 6-month-old kittens. Furthermore, FeLV-infected kittens became non-viremic after treatment with antibodies to the murine virion glycoprotein³. This finding indicates that the interspecies portions of C-type virus glycoproteins can be suitable targets for immunotherapeutic regimens. It was of interest to extend the studies with leukaemia viruses to agents which induce other classes of neoplasms. We describe here a study of the effect of antibody therapy on infections in cats with feline sarcoma virus (FeSV). Our preliminary results indicate that antibody therapy can be effectively applied for prevention of virus-induced solid tumours. Moreover, this tumour model may be more attractive and amenable to study than virus-induced leukaemia.

The virus used in these studies was the Snyder-Theilen strain of FeSV. Kittens were injected with aliquots from a single pool of cell-free tumour extracts containing between $10^{3.2}$ and $10^{4.2}$ focus inducing units (FIU) representing about 10^2 and 10^3 cat LD₅₀ units respectively.

The antisera used have been described in detail elsewhere^{2,3}. Briefly, goat antiserum to FeLV ('FeLV serum') neutralised the homologous virus to a high titre (1/8,000) and was highly cytotoxic in a Cr-release test for FeLV producing F422 cells (titre 1/8,000). This serum was absorbed with foetal calf serum and normal cat antigens before use. Goat antiserum to MuLV Friend gp71 ('MuLV gp71 serum'), also prepared in goats, neutralised FeLV to a titre of 1/400 and was cytotoxic for F422 cells to a titre of 1/500. Total globulins of both sera were obtained by precipitation with ammonium sulphate. The IgG fraction of MULV gp71 serum was then isolated by chromatography on DE-52. The respective globulin fractions and MuLV gp71 IgG were used at a concentration of $5 \times$ with respect to whole serum volume.

Three-week-old kittens from a specific pathogen-free colony were infected subcutaneously (s.c.) in the scapular region with the cell-free FeSV tumour extract. Six or 12 d later, whole serum, globulins or IgG were administered once intraperitoneally followed by seven s.c. injections over a period of 16 d (see Table 1). In Experiment I, all kittens were from the same mother, whereas the other studies were with randomised animals.

As can be seen from the effect on the 11 control animals, represented by untreated animals or groups which received normal goat serum globulins, the FeSV tumour extract was lethally oncogenic in our system. All animals died after development of progressive sarcomas with many secondary growths which probably, in large measure, represent new primaries induced by FeSV. The mean survival period for kittens infected with $10^{3.2}$ FIU was 53 ± 9.4 d, whereas the animals receiving the higher virus dose died somewhat earlier.

Of the two antibody treatments employed, the outcome with FeLV-serum globulins was more effective. When the

Table 1

Exp. No.	Virus dose (FIU)	No. of cats	Treated with	Amount (ml) injected; days p.i.	Tumour detected at days p.i.	Tumour growing to size of	Complete regression at weeks p.i.	Survival period of cat (days)	Number and of animals recovered	%
I	10 ^{4.2}	2	FeLV serum globulin	11 ml 6-22	(a) 15 (b) 15	Hazelnut Hazelnut	4 4	> 285 90†	2 (100)	
		2	Normal goat globulin	11 ml 6-22	(a) 23 (b) 23	P with S*	NA†	30 28	None	
II	10 ^{3.2}	5	FeLV serum globulin	11 ml 6-22	No Tumour	No Tumour	NA	all > 211	5 (100)	
		4	Normal goat globulin	9 ml 6-22	(a) 20 (b) 38 (c) 36 (d) 30	P with S " " "	NA " " "	47 63 55 48	None	
		3	—	—	(a) 34 (b) 20 (c) 30	P with S " "	NA " "	60 51 37	None	
		1	FeLV serum globulin	11 ml 12-28	No Tumour	No Tumour	NA	> 110	1 (100)	
		5	MuLV gp71 Serum IgG	11 ml 6-22	(a) No Tumour (b) 30 (c) 19 (d) 28 (e) 30	No Tumour Hazelnut P with S " "	NA 6 NA " "	> 110 49 41 41	2 (40)	
IV	10 ^{3.2}	2	Normal goat serum IgG	11 ml 6-22	(a) 48 (b) 50	P with S "	NA "	50 68	None	

* P with S—progressive with secondary growths.

† NA—not applicable.

‡ This animal died of enteritis; no tumour evident.

treatment was initiated 6 d post-infection (p.i.), none of the animals died as a consequence of tumour development. The single animal in Experiment I which died 90 d p.i. was lost due to enteritis, and showed no evidence of tumour. In Experiment III, where only one kitten was tested, no tumour developed even when the antibody treatment was initiated as late as 12 d p.i.

Kittens in Experiment I, receiving the higher virus dose, developed clearly palpable tumours at the site of FeSV inoculation during antibody treatment. In contrast to the situation in the control animals these tumours completely regressed. After antibody treatment, scars remained in the skin at the site of virus inoculation which were surrounded by pigment-free hairs (Fig. 1b). This occurred regardless of whether palpable tumours were detected. Thus far, no indication has been obtained that any of the surviving animals have developed new tumours (in Experiment I this represents nearly 285 d p.i.).

Some protection against FeSV tumour development was also achieved with the MuLV gp71-IgG, although in this study (Experiment IV), only two of five kittens survived. One of the surviving animals developed a visible tumour which subsequently regressed. Thus, interspecies MULV gp71 antibody can be effective against solid tumours induced by FeSV. Additional studies using larger amounts of IgG and other protocols are in progress to improve the effect of the treatment.

Our previous studies in kittens cured of FeLV infection by antibody treatment revealed that the non-viremic animals resisted subsequent challenges with FeLV and developed significant neutralising titres against the virus³. Some of these animals received three further challenges with live FeLV and one with FeSV. One female cat from this group of hyperimmunised animals was mated and subsequently delivered three kittens which were challenged with $2 \times 10^{3.2}$ FIU of FeSV tumour extract at 22 d of age. These animals had not developed any palpable tumours at 70 d p.i.

The prime features of these preliminary studies can be

Fig. 1 a, Tumour of a control cat infected with 10^{3.2} FIU of feline sarcoma virus: 60 d p.i. b, Scar of a cat infected with 10^{3.2} FIU and treated with FeLV serum globulin: 43 d p.i.



summarised as follows: (1) Protection against death from development of progressive FeSV-induced tumours can be achieved by administration of globulin fractions from goats immunised with (a) FeLV and (b) MuLV gp71. (2) In some cases, animals protected by the antibody treatment developed sizeable tumours at the site of virus inoculation. But, these tumours regressed while control cats died of progressive sarcomas. (3) Scar formation at the site of FeSV inoculation was observed in several cases in recovered animals. (4) Preliminary results indicate that transfer of immunity from mother to offspring may be possible.

The system described above seems to represent a classical model for study of tumour growth and regression in the cat in response to external immunological controls. It is certainly remarkable that by simple application of antibody, it is possible to achieve such a striking effect on a solid tumour without any obvious detriment to the host. Of particular interest are the cases where the tumour was detectable by palpation during the course of antibody treatment, suggesting that a direct interaction of the heterologous antibody with the growing tumour may occur. The actual mechanism by which the tumour is eliminated and the significance of the residual scars are under investigation. Our previous experience in the mouse and cat indicates that an immune response by the host was required for long-term protection against virus infection and/or leukaemia development. In the present system, such an immune response could be directed against prominent cell surface antigens including virus structural components and/or FOCMA (ref. 4). Further studies are required to determine if such host immunity is in fact activated, and whether it is sufficient to guarantee long-term protection against sarcoma. The transfer of immunity from mother to offspring reported also by others in the cat^{5,6} as well as in the mouse^{7,8} is an important phenomenon. The nature and specificity of the antibodies primarily involved, however, are not yet known.

The results presented above together with other studies in the mouse and cat are highly promising and suggest possible application of antibody therapy to other virus-induced malignancies. Of special interest in this regard is the interspecies activity of the antibody *in vivo*. This raises the possibility that tumours in widely differing species expressing cross-reactive glycoprotein antigens will be amenable to similar studies, using well-characterised reagents such as anti-MuLV gp71 antisera.

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FERNANDO DE NORONHA
RAYMOND BAGGS

Department of Veterinary Pathology,
New York Veterinary Medicine,
Cornell University, Ithaca, New York 14850

WERNER SCHÄFER

Max-Planck-Institut für Virusforschung,
Tübingen, FRG

DANI P. BOLOGNESI

Department of Surgery,
Duke University Medical Center,
Durham, North Carolina 27710

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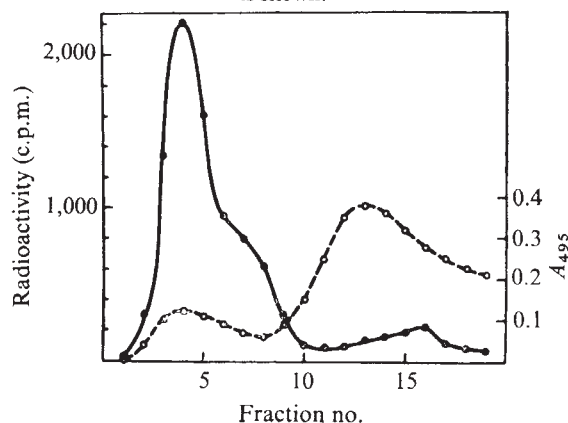
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Melanotropin-daunomycin conjugate shows receptor-mediated cytotoxicity in cultured murine melanoma cells

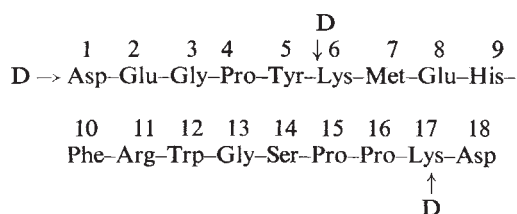
MELANOTROPIN (MSH) binds to specific receptors at the surface of mouse melanoma cells, preferentially in the G-2 phase of the cell cycle¹. On prolonged incubation, a fluorescein-MSH conjugate is internalised and appears intracellularly in small vesicles². The same phenomenon has been demonstrated by electron microscopy with an MSH-ferritin-fluorescein conjugate³. Although a role for the internalisation of MSH receptors in the execution of the hormonal message is still open to question, the fact that these conjugates of MSH are specifically recognised and internalised by melanoma cells offers a possibility for specific, site-directed chemotherapy of melanomas. We have therefore assumed that MSH-toxin conjugates might be specifically recognised and internalised and thereby have a selective toxic effect on cells that display MSH receptors on their surface. Even though the principle of site-directed chemotherapy has been known for a long time, it is only recently that this field became active⁴. Conjugates of specific antibodies and cytotoxic substances⁵⁻⁷, as well as a DNA-daunomycin complex⁸ have been used in an attempt to increase the selectivity of the toxic agents. In this report we describe the synthesis of a melanotropin-daunomycin conjugate and its MSH receptor mediated cytotoxic effect on mouse melanoma cells *in vitro*.

A melanotropin-daunomycin conjugate was prepared using the procedure developed by Hurwitz *et al.*⁹ for the covalent binding of daunomycin to antibodies. Daunomycin 20 mg (380 μ mol) (Sigma) was dissolved in 1.5 ml phosphate-buffered saline, pH 7.4 (PBS). NaIO₄ 11.5 mg (540 μ mol) was added and the mixture was incubated at 22 °C in the dark for 1 h. The excess NaIO₄ was neutralised with glycerol, added to a 50 mM final concentration. 30 mg β -MSH (prepared by us¹⁰) containing 1 μ Ci of ¹²⁵I-MSH (1) as a marker was added in 1 ml of 0.15 M K₂CO₃ (pH 9.5). The mixture was incubated for 1 h at 22 °C. The resultant conjugate was reduced by NaBH₄ (0.3 mg ml⁻¹) at 37 °C for 2 h. The reaction mixture was applied to a Sephadex G-25 (90 cm $\times$ 2 cm) column and eluted with PBS. Radioactivity of the fractions was measured by a γ -counter and the concentration of daunomycin was monitored spectrophotometrically at 495 nm. The appearance of the red colour in the void volume, coincident with the major peak of ¹²⁵I-MSH indicated the presence of a melanotropin-daunomycin conjugate (Fig. 1). On the basis of

Fig. 1 Separation of melanotropin-daunomycin on Sephadex G-25. A reaction mixture of melanotropin-daunomycin was applied to a Sephadex G-25 column as described in the text. Radioactivity ($\bullet$) and absorbancy at 495 nm ($\circ$) of the fractions is shown.



the specific radioactivity and absorbancy at 495 nm of the major fraction, the molar ratio of daunomycin to MSH in the conjugate was calculated to be 2.2:1. The purity of the compound was tested by high voltage electrophoresis. The purified conjugate was hydrolysed by 6 N HCl at 110 °C for 18 h and the amino acid composition was determined, parallel with the hydrolysate of a mixture of daunomycin and unconjugated β -MSH. About 50% of lysine and aspartic acid were recovered from the conjugate indicating that the oxidised product of daunomycin (D) had substituted the ϵ -aminolysyl residue(s) (no. 6 and/or no. 17) and the terminal amino group of aspartic acid. This suggests the following general formula for the melanotropin–daunomycin conjugate:



Efforts to delineate substitution of lysyl residues by analysis of tryptic and chymotryptic hydrolysates were unsuccessful.

The cytotoxic index of daunomycin was 0.99 when the antibiotic was added in 4 μM concentration to cultures of melanoma cells. Melanotropin–daunomycin showed the same level of toxicity at 1.4 mM (Table 1). On a molar basis, therefore, the daunomycin–MSH conjugate is about three times more toxic to melanoma cells than free daunomycin. MSH, daunomycin and melanotropin–daunomycin conjugate markedly changed the morphology of melanoma cells. With daunomycin, cells develop large nuclei, most probably due to a G-2 block, similar to that observed in other systems¹¹; surviving cells showed no dendritic processes. In the presence of melanotropin–daunomycin, mostly cells with large nuclei were found with normal or fragmented processes. The median cell volume in the control cultures was 1,450 μm^3 ; in the presence of MSH 1,500–2,000 μm^3 ; and with melanotropin–daunomycin, 1,700–2,600 μm^3 . Although free daunomycin was as toxic to 3T3 cells as to melanoma cells, melanotropin–daunomycin (1.4 μM) was not toxic to 3T3 fibroblasts (Table 1). Neither MSH (0.2 mM)

nor MSH–daunomycin (1.4 μM) had significant effect on the morphology or size of 3T3 cells. The toxic effect of melanotropin–daunomycin can be prevented by free MSH; free MSH has no effect on the toxicity of daunomycin (Table 1).

Daunomycin or melanotropin–daunomycin conjugate was added to coverslip cultures of melanoma cells and of 3T3 cells and the uptake of daunomycin was monitored by fluorescence microscopy. After 1 h of incubation at 37 °C, internalisation of daunomycin was detected by fluorescence microscopy with both melanoma and 3T3 cells (Fig. 2a,b). The daunomycin–melanotropin complex was internalised by melanoma cells only (Fig. 2c, d) and this process could be blocked by free melanotropin (Fig. 2e). Melanotropin had no effect on the uptake of daunomycin by melanoma cells (Fig. 2f) or by 3T3 cells (not shown).

Thus we have synthesised a melanotropin–daunomycin conjugate which is more toxic to mouse melanoma cells than daunomycin alone and in the same concentration range the conjugate is not toxic to mouse 3T3 fibroblasts. This increase in selective toxicity is remarkable because unconjugated daunomycin is equally toxic to both cell lines (Table 1); the same cytotoxicity of free daunomycin was found in other systems^{8,9}. Because both the toxic effect and uptake of melanotropin–daunomycin in melanoma cells can be partially prevented by free melanotropin (Table 1 and Fig. 2), we assume that recognition of melanotropin–daunomycin by MSH receptors, present on melanoma cells¹ but absent from 3T3 cells (unpublished work), is responsible for the enhanced selective toxicity.

As we could demonstrate by fluorescence microscopy, after 1 h of incubation, the fluorescence of unconjugated daunomycin (or the fluorescence of the aglycone) appears in the nuclei of both melanoma and 3T3 cells.

In contrast, melanotropin–daunomycin appears in melanoma cells only. Whether the conjugate reaches the nucleus intact or whether it is degraded, is not known. The mechanism of penetration of melanotropin–daunomycin into melanoma cells is probably similar to the endocytosis we observed with fluorescein–MSH (ref. 2) and with fluorescein–ferritin–MSH (ref. 3). These conjugates as well as ¹²⁵I – MSH (ref. 12) bind in patches on the surface of melanoma cells at 0 °C (refs. 2, 3, 12). At 37 °C, FITC–MSH appear in vesicles² and the MSH–ferritin–FITC conjugate in premelanosomes in a few hours. We assume that endocytosis of these conjugates resembles phagocytosis by

Table 1 Toxicity of melanotropin–daunomycin on melanoma and 3T3 cells

Cells	Additions to culture medium	Cytotoxic index*
Melanoma	None	0.00
	Daunomycin, 4 μM †	0.99
	Daunomycin, 4 μM + melanotropin, 200 μM	1.00
	Melanotropin, 2 μM	0.05
	Melanotropin–daunomycin, 0.35 μM	0.65
	Melanotropin–daunomycin, 0.70 μM	0.87
	Melanotropin–daunomycin, 1.40 μM	0.98
	Melanotropin–daunomycin, 1.40 μM + melanotropin, 4 μM	0.57
	Melanotropin–daunomycin, 1.40 μM + melanotropin, 40 μM	0.47
	Melanotropin–daunomycin, 1.40 μM + melanotropin, 200 μM	0.28
3T3	None	0.00
	Daunomycin, 4 μM †	0.99
	Melanotropin, 2 μM	0.01
	Melanotropin–daunomycin, 1.4 μM	0.02

Cloudman S-91 melanoma cells (NCTC 3960) were inoculated in 25 ml Falcon flasks and cultivated at 37 °C in an atmosphere containing 5% CO₂, in Ham's F-10 medium supplemented with 5% foetal calf serum and 2 U ml⁻¹ gentamycin. The mouse 3T3 fibroblast cell line (from Dr Paul Lebowitz) was cultivated in Earle's MEM containing 5% foetal calf serum, 100 U ml⁻¹ penicillin and 100 U ml⁻¹ streptomycin. After 1 d of growth, the medium was changed to a fresh one containing additions as listed. After 3 d in culture, the cells were rinsed with fresh medium and removed from the flasks by shaking, after treatment with EDTA in Tyrodes solution⁴. The cells were pelleted by centrifugation (1,000g, 5 min.) and washed with serum-free medium. The number and size of the cells was determined with a Coulter 'channelyser'. Cell viability was determined by a modification of the dye exclusion test¹⁴. Four parallel flasks were used for each point.

*Cytotoxic index = $\frac{(\text{no. viable cells in control culture} - \text{no. viable cells in treated culture})}{(\text{no. viable cells in control culture})}$

†Minimum dose of daunomycin required for maximum cytotoxicity. This was determined from dose–response curves and the values for melanoma and 3T3 cells were similar to those observed with other cell types^{8,9}.

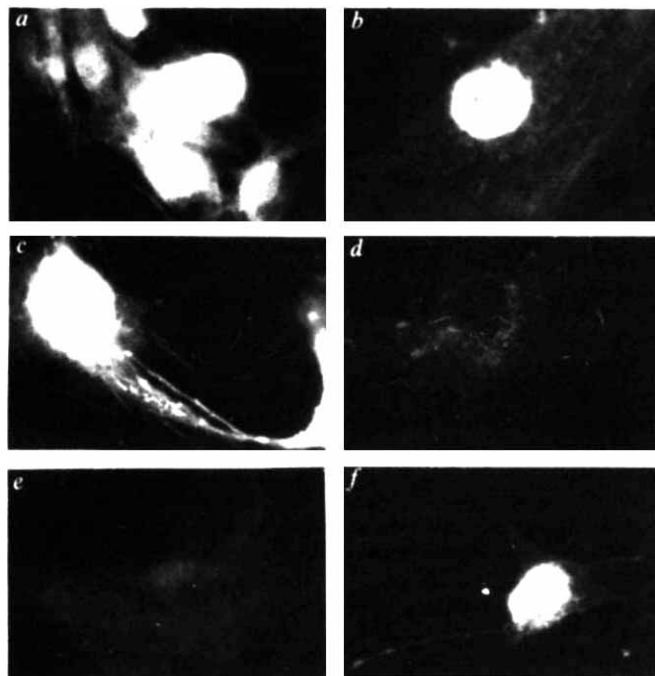


Fig. 2 Fluorescence microscopy of daunomycin and melanotropin-daunomycin treated cells. Melanoma cells and 3T3 fibroblasts were grown for 2 d in Ham's F-10 medium on coverslips. Before treatment, the cultures were rinsed with fresh medium; then incubated at 37 °C for 60 min as follows: *a*, melanoma cells were incubated with 4 μM daunomycin; *b*, 3T3 cells with 4 μM daunomycin; *c*, melanoma cells with 1.4 μM melanotropin-daunomycin; *d*, 3T3 cells with 1.4 μM melanotropin-daunomycin; *e*, melanoma cells with 1.4 μM melanotropin-daunomycin + 0.1 mM melanotropin; *f*, melanoma cells with 4 μM daunomycin + 0.1 mM melanotropin. After incubation, cells were rinsed with serum-free medium (0 °C), fixed with 2% paraformaldehyde for 5 min (0 °C), rinsed with distilled water and air-dried. Preparations were mounted with Leitz immersion oil and cellular localisation of the red fluorescence (white areas on the microphotographs) due to the presence of free daunomycin or melanotropin-daunomycin was detected by fluorescence microscopy by using a Leitz Diavert microscope equipped with a 100 W mercury burner and a Ploem epi-illuminator.

macrophages¹³ suggesting that MSH receptors may play a similar role on melanoma cells as Fc receptors do on macrophages: the formation of a ligand receptor complex may signal endocytosis. Accordingly, the absence of MSH receptors and permeability barrier to MSH in 3T3 cells would explain lack of endocytosis of the hormone-toxin conjugate which may be responsible for the resistance of 3T3 cells to melanotropin-daunomycin.

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J. M. VARGA
N. ASATO
S. LANDE
A. B. LERNER

Department of Dermatology,
Yale University School of Medicine,
New Haven, Connecticut 06510

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Procarcinogen activation and hormonal control of cell proliferation in differentiated primary adult rat liver cell cultures

CONCERTED interactions among hormones and nutrients control animal cell proliferation¹. For example, in the rat, insulin, glucagon, glucocorticoid, parathyroid hormone, iodothyronines and amino acids regulate normal hepatic proliferation—a prominent system for studying epithelial cell growth control². Endocrine and nutritional factors also modify the efficiency with which chemicals produce experimental tumours in these animals notably, the induction of hepatomas by aromatic amines³. We report here observations with a novel primary monolayer adult rat liver culture system which may prove helpful towards studying these interactions under defined conditions⁴. These cells proliferate in response to hormones and nutrients; they maintain differentiated hepatic functions such as albumin and arginine biosynthesis for at least 8-10 days after plating; and they metabolise the procarcinogen *N*-2-acetylaminofluorene (AAF) into a proximate form, *N*-hydroxy-AAF (ref. 5).

Cell isolation procedures and plating methods (which use arginine-free medium to select for arginine-synthesising hepatocytes⁶) are given in Fig. 1 (legend). About 10% of the cells in 5-7 g of normal liver are recovered for plating. Over 95% of these cells are viable (measured by trypan-blue exclusion) and hepatocyte-like with respect to size (≥ 25 μm diameter, measured with an electronic particle counter), to histochemical staining properties (positive for glycogen and glucose-6-phosphatase), and to ultrastructure visualised by electron microscopy (glycogen granules, microbodies, rough endoplasmic reticulum; K. Dempo *et al.*, in preparation). Similar characteristics are retained 24-48 h post-plating by the surviving cell fraction, attached to the plastic substratum, which represents about 10-20% of the initial inoculum and which begins to form monolayer aggregates⁶ at this time (see ref. 4 for photomicrographs). The recovery efficiency⁶ of 10-20% is invariant with inoculum concentrations of 10^5 - 10^6 cells per dish. Cell survival after 72-96 h requires serum (Fig. 1a) without which cells randomly detach (floating cells are dead and eventually most lyse).

Growth curves are shown in Fig. 1. Serum alone fails to sustain significant proliferation rates but if hormones and inosine⁷ are added, especially hydrocortisone or insulin, proliferation is stimulated (Fig. 1a). Titration studies suggest synergistic hormone interactions; stimulation is detected at concentrations as low as 50 ng ml⁻¹. Analogues with negligible potency (for example desAla, desAsp-insulin⁸ or androstenedione⁹) are non-stimulatory. Net increased proliferation, which absolutely requires exogenous ornithine, is otherwise satisfied by 0.4 mM arginine but not by either exogenous putrescine (0.008-0.4 mM) or 8 μM arginine (Fig. 1b). DNA synthesis rate and mitotic kinetic patterns are similar to those reported for foetal hepatocytes¹⁰. Under optimal conditions (Fig. 1a), adult log-phase cultures (96-120 h post-plating) contain at least $46 \pm 2\%$ (s.e.m.; $N = 3$) of cells in S-phase, measured radioautographically¹⁰ following a 16-h pulse with ³H-dT or as estimated by flow microfluorimetric methods⁷.

Similar growth curves are obtained if 24 h cultures receive fresh 24 h cell-free 'conditioned medium'; therefore, enhanced attachment or cell-settling do not cause increased cell numbers.

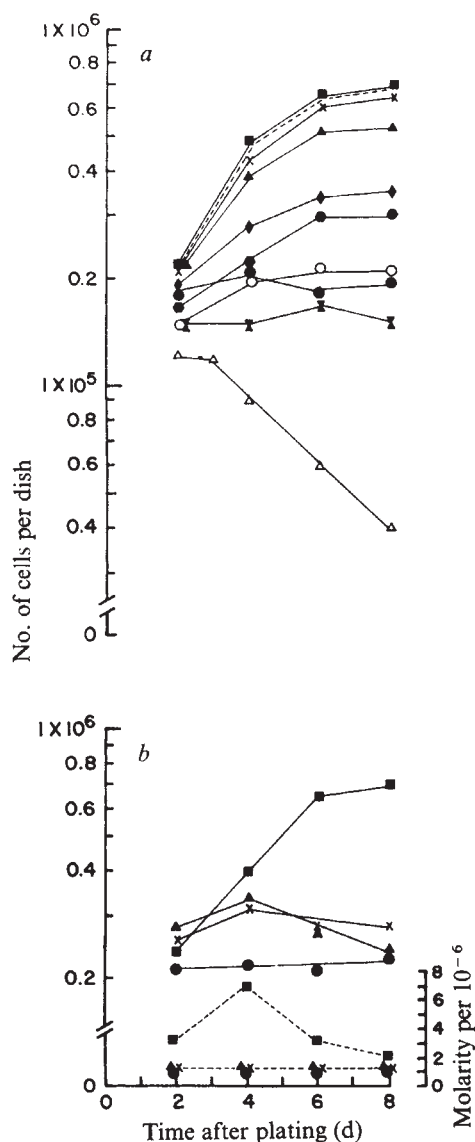


Fig. 1 Hormonal control of cell proliferation in adult liver cell cultures. Fisher/344 male or female rats (150–200 g) were ether anaesthetised at 0900–1200 h. An abdominal incision was made extending into the thoracic cavity; the rat was exsanguinated by cardiac puncture and its intestinal contents resected. A 20 ml plastic syringe equipped with a 25 gauge $\times$ 1 inch needle was inserted into the portal vein, clamped distally to splanchnic and gastric veins, and the superior vena cava was cut. The liver was perfused 2–3 min with 20 ml 'digestion buffer.' This solution contains (in g l⁻¹) Type I Sigma collagenase, 3.0; NaCl, 7.597; KCl, 0.224; NaH₂PO₄·H₂O, 0.138; D-glucose, 1.802; phenol red, 0.0012; insulin (from W. Bromer, Eli Lilly), 0.01; and hydrocortisone-succinate and inosine (Sigma), 0.01 each: it is prepared fresh, adjusted to pH 7.4, sterilised with a 0.45 μm Nalgene filter, and preincubated 1–4 h at 37 °C. Two perfused excised livers were freed of extraneous tissue and washed twice with 10 ml 'complete medium' (arginine-free Dulbecco-Vogt's modified Eagle's medium, supplemented with 0.4 mM L-ornithine, 15% v/v heat inactivated dialysed foetal bovine serum⁶ and 10 μg ml⁻¹ each of glucagon, T₃ and of hormones and purine present in digestion buffer). 'Basal medium' is complete medium minus hormone and purine supplements. Tissue was dissected into 5 $\times$ 8 mm pieces, washed twice, pooled and incubated at 37 °C with 40 ml aliquots of digestion buffer in a 125 ml Erlenmeyer flask (four 10-min treatments; 5 cm $\times$ 7 mm magnetic stirring bar and Magne-Stir Model No. 10 stirrer, rheostat control 35–40). Cell suspensions after each treatment were transferred to a collecting flask containing 10 ml 'complete medium' at 0–4 °C. Ice-cold medium (160 ml) was mixed into the final pooled cell suspension; 40 ml aliquots were dispersed into eight 50 ml tubes (Falcon) and centrifuged at 150–200g for 13 s. Pellets were gently resuspended in serum-free 'complete medium' and recentrifuged. Resulting pellets were vigorously resuspended and titrated with a Coulter counter (threshold > 10 μm ; attenuation, 1; aperture, 256). A third centrifugation step was repeated and the final pellets resuspended into appropriate plating media. Cells were plated (10⁶ per 2 ml medium per 35 mm Falcon plastic tissue culture dish) and cultured, without media changes, in an humidified 10% CO₂-air incubator at 37 °C. Cell counts (numbers of attached cells removed by trypsin) and amino acid analyses were performed by previously validated methods^{6,16}. Counting errors were $\pm$ 2–10% ($N = 3$). *a*, Cell growth curves under various plating conditions: complete medium, male (—■—) or female cells (—●—). All other cells were from males with basal medium (—●—), basal medium plus 10 μg ml⁻¹ L-T₃ (—■—), or glucagon (—○—) or inosine (—◇—), or insulin (—◆—) or hydrocortisone (—▲—) or inosine and insulin and hydrocortisone (—×—); complete medium minus serum (—△—). *b*, Growth curves (solid lines) of cells plated in complete medium $\pm$ 0.4 mM L-arginine (—■—); without L-ornithine (—●—); or with ornithine replaced by 8 μM arginine (—×—) or by 0.008–0.4 mM putrescine (—▲—). Free arginine levels in culture media (except +0.4 mM arginine condition) are shown by dashed lines.

Serum contains additional stimulatory material for if slowly growing cultures are established in 'complete medium' with 1% serum, then increasing amounts of serum added 24–48 h post-plating produce a dose-dependent family of increasing growth curves (not shown). Triiodothyronine or glucagon (Fig. 1a), and FGF (ref. 11), or EGF (ref. 12) (0.01–1.0 μg ml⁻¹) do not stimulate growth and thus do not seem to be limiting under these conditions (see discussion in ref. 13). Although cell proliferation in this adult system is more dependent than a similar foetal system upon exogenous ornithine¹⁴ and hydrocortisone¹, it is not yet clear if this results from different intrinsic cellular properties and/or from different initial physiological states.

³H-ornithine is a useful probe of specific and general metabolic functions in liver cell cultures⁶ (Fig. 2). These studies indicate that cultured adult cells synthesise albumin and arginine because first, ³H-ornithine enters albumin and cellular macromolecules which, after acid hydrolysis, contain >80% of the protein-associated radioactivity in the form of ³H-arginine (not shown), and second, incorporation is inhibited $\geq$ 98% by cycloheximide (5 μg ml⁻¹; not shown). The immunoprecipitation procedure used¹⁵ to measure albumin biosynthesis accurately reflects relative rate differences of albumin production because an independent previously validated radioimmunoassay¹⁶ gives similar results to those shown in Fig. 2. Thus, day 2 and 8 rates measured by radioimmunoassay are 110 and

260 ng 'albumin' per 10⁶ cells per 6 h, respectively, whereas these rates measured by immunoprecipitation are 60,000 and 140,000 ³H-labelled 'albumin' c.p.m. per 10⁶ cells per 6 h. ³H-ornithine uptake is liver-specific because it is arginine-suppressible (Fig. 2) and detectable radioautographically as cytoplasmic grains⁶, mainly in monolayer aggregates⁴. Cells possessing this function (ornithine transcarbamylase) comprise 74 $\pm$ 3% (s.e.m.; $N = 3$) of the attached cell fraction 96–120 h post-plating. If log-phase cultures are labelled with both ³H-dT (as above) and ³H-ornithine (Fig. 2), radioautographic analyses show that 40 $\pm$ 2% (s.e.m.; $N = 3$) of ornithine-labelled cells also are in S-phase. Therefore, a substantial fraction of identifiable parenchymal hepatocytes traverse the 'cell-cycle' in this system. The extent to which DNA-synthesising cells not detectably labelled with ³H-ornithine (about 33% of the S-phase population) represent dedifferentiated hepatocytes and/or a contaminant population cross-fed via cell-cell interactions¹⁶ is as yet unknown.

Figure 2 also shows parallel increases in rates of ³H-ornithine uptake into cellular 'protein' and into released ³H-albumin during log growth; during stationary phase, these rates decrease to 30–40% and to about 80% of maximal, respectively. Moreover, arginine suppresses ³H-ornithine uptake into cellular 'protein' more efficiently than into released ³H-albumin. These observations could underlie intracellular compartmentalisation

of amino acid pools used for different protein synthetic processes in this system. This interpretation is complicated, however, by our inability rigorously to exclude the presence of heterogeneous cell types. Additional hepatic parenchymal cell functions present in this system include α_1 -foetoprotein, urea, tyrosine, and very low density lipoprotein biosynthesis (H. Leffert *et al.*, in preparation), and the hepatic microsomal hydroxylases¹⁷ (Fig. 3).

Conversion of ^{14}C -AAF into $N\text{-OH-}^{14}\text{C}$ -AAF (which is released into the medium) is shown in Fig. 3. This process appears liver cell-specific (e.g., primary adult rat pituitary or 3T3 cells lack this function) and is performed by viable cells, not by extracellular chemical and/or enzymatic mechanisms. Intracellular metabolism of $N\text{-OH-}^{14}\text{C}$ -AAF is suggested⁵ because cell-associated radioactivity, detected as 5% trichloroacetic acid-insoluble material, can be solubilised by trypsin, or KOH, or DNase treatment; moreover, unlabelled AAF specifically competes for ^{14}C -AAF uptake; and conversion is modified by gonadal steroids (H. Leffert *et al.*, in preparation). Relative proportions of hydroxylated metabolites released *in vitro* ($N\text{-OH-AAF} = 2\%$, 3-, 5-, 7-, and 1-OH-AAF = 17, 15, 7, and 1%, respectively) simulate urinary metabolite distributions reported for male rats fed ^{14}C -AAF (ref. 18). This determination was independently confirmed using a second solvent system which, in addition to resolving $N\text{-OH-}^{14}\text{C}$ -AAF,

Fig. 2 Albumin production and ^3H -ornithine uptake in adult liver cell cultures. Male cultures were established using complete medium (see Fig. 1). Media were aspirated at various times, the dishes washed twice with 2 ml complete medium (minus ornithine) and then fed 2 ml similar medium containing 25 μCi ^3H -3-dl-ornithine (1 mCi per 68 μg ; New England Nuclear) without (open symbols) or with (shaded symbols) 0.4 mM arginine for 6–8 h. Harvested fluids were pretreated with an unrelated antigen-antibody system, to remove nonspecific radioactivity adventitiously adsorbed to specific immune-complexes, and then assayed for ^3H -labelled rat albumin ($\square$ — $\square$) by immunoprecipitation with purified monospecific goat anti-rat albumin antibody¹⁵. About 20% of the total radioactive material present in the culture fluids was acid-insoluble; of this, 20–30% was detected as 'albumin' either by the above procedure or by isoelectric focusing together with authentic rat albumin¹⁵. Culture monolayers were assayed¹⁴ for precursor uptake into acid-insoluble material ($\circ$ — $\bullet$). A similar pattern is observed if uptake into 90 $^\circ\text{C}$ (20 min) heat-stable acid insoluble material is determined¹⁴; about 40% of the counts are solubilised at any time under these conditions. Measurement errors were $\pm 10\%$.

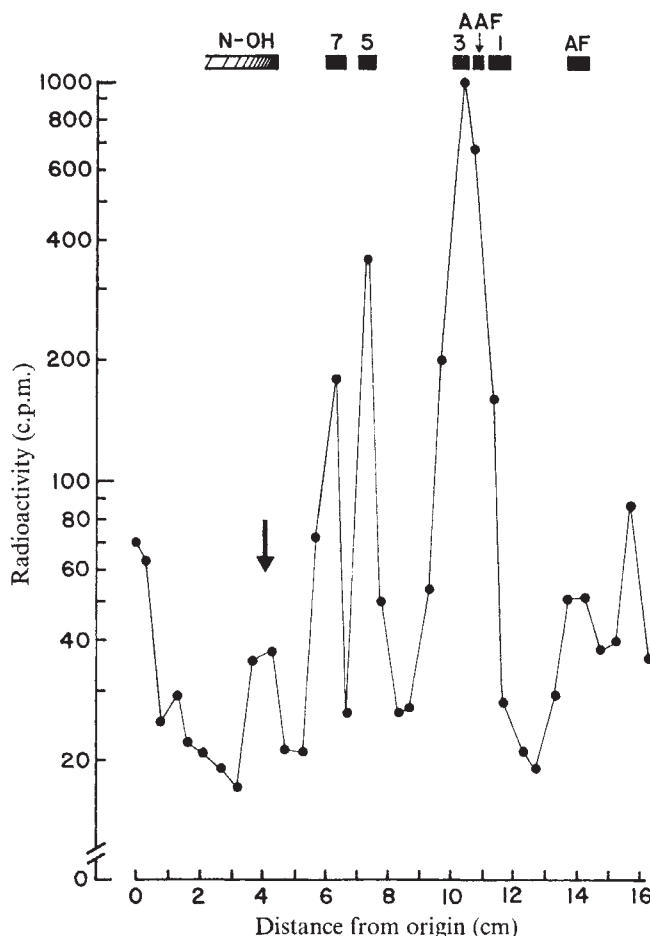
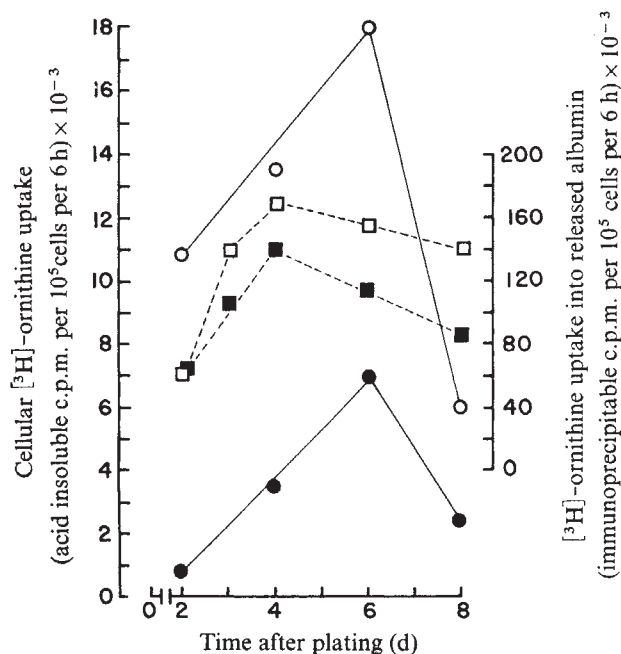


Fig. 3 N -hydroxylation of 2-acetylaminofluorene by cultured adult liver cells. Male cultures 5–6-d-old (Fig. 2) were treated 48 h with 20 μl of a 95% v/v ethanol solution of ^{14}C -9-AAF (0.1 mCi per 0.55 mg; 20 $\mu\text{Ci ml}^{-1}$; 100 $\mu\text{g ml}^{-1}$ (New England Nuclear); 96–98% of the radioactivity is found in the medium after 48 h. Two ml cell-free culture medium was added to 3 ml 1 M sodium acetate buffer, pH 6.0, containing 50 μl CHCl_3 and 2.5 mg each of β -glucuronidase and α -amylase (61 and 55 U mg^{-1} , respectively; Sigma)²⁰ and incubated for 24 h at 37 $^\circ\text{C}$. Acid-washed ether extracts of the resulting solution, which removed about 65% of the counts, were chromatographed^{19,20} on silica gel 60 plates 5 $\times$ 20 cm; Brinkman) using benzene/acetone (8/2) and developed four times; recovery was $\geq 98\%$. Radioactivity present in 5 $\times$ 0.5 cm strips scraped into plastic vials containing 10 ml 'cocktail' (3.79 l toluene, 160 ml Liquefluor, 158 g Cab-O-Sil (Eastman Kodak) was detected by scintillation counting with measurement errors of $\pm 0.2\%$. R_f values of authentic $N\text{-OH-AAF}$ (cross-hatched rectangular bar), ring-hydroxylated metabolites, ^{14}C -AAF and aminofluorene (AF) (solid bars) are indicated above radioactive profile (corrected for background of 8 c.p.m.). Standards were detected with Folin/ Na_2CO_3 and p -nitro-methylbenzaldehyde reagents¹⁹. Peak fractions (arrow indicates position of $N\text{-OH-}^{14}\text{C}$ -AAF) were confirmed identical with authentic standards by rechromatography using a second system (chloroform/methanol, 97/3; development three times). Radioactivity in both systems which cochromatographed with authentic $N\text{-OH-AAF}$ was five to eightfold greater than background.

separates 1- and 3-position ring-hydroxylated metabolites from AAF (see legend to Fig. 3)^{19,20}.

The critical interactions required of hormones, nutrients and carcinogens (at cellular and subcellular targets) to alter proliferative control are as yet unknown. This system may provide a useful tool to study this problem.

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H. L. LEFFERT
T. MORAN
R. BOORSTEIN
K. S. KOCH

Cell Biology Laboratory,
The Salk Institute,
Post Office Box 1809,
San Diego, California 92112

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Activation of human leukocyte elastase by human α_2 -macroglobulin

PROTEOLYSIS and its biological control in the pathogenesis of disease states involving inflammation and tissue injury have become areas of intensive study^{1,2}. Because of their probable involvement in the pathogenesis of emphysema, the elastolytic enzymes derived from neutrophils have received particular attention^{3–6}. Among the principal protease inhibitors in blood, α_1 -antitrypsin (α_1 -AT) serves as the main inhibitor controlling the activity of elastase and other serine proteinases, whereas α_2 -macroglobulin (α_2 -M) is believed to function primarily as a means of removing elastase and other proteinases from the circulatory system via the reticuloendothelial system^{7,8}. During studies of the interaction of various inhibitors with elastase isolated from purulent sputum⁹, it was noted that the activity of this enzyme towards certain synthetic substrates was markedly enhanced in the presence of α_2 -M. We present here a study of this activation. It is possible that activation of elastase by α_2 -M may be of physiological significance, particularly under those conditions where a deficiency of α_1 -antitrypsin is known to be a predisposing factor towards chronic obstructive pulmonary disease¹⁰.

Leukocytic elastase was prepared from purulent sputum⁹ and was comprised of three isozymes with no other impurities detectable by gel electrophoresis. For purposes of calculation, however, this mixture of enzymes was assumed to be a single entity with a molecular weight of 27,000 (ref. 9). human α_2 -M was prepared from plasma according to Harpel¹¹ and was judged to be at least 98% homogeneous by gel electrophoresis¹². Its molecular weight, as determined by sedimentation equilibrium, was 726,000. Although the activity of the elastase towards casein was almost completely inhibited by a twofold molar excess of α_2 -M, its activity towards the synthetic substrate Suc-(Ala)₃-Nan (*N*-succinyl-L-alanyl-L-alanyl-L-alanine-4-nitroanilide) was dramatically enhanced in the presence of α_2 -M (Fig. 1). Extrapolation of the linear segments of the curve in Fig. 1 shows maximum activation (15-fold) to occur when the weight ratio of α_2 -M to elastase is approximately 12 to 1. Based on the molecular weight values referred to above, this weight ratio corresponds to a molar ratio of 1:2.2. From Lineweaver–Burk plots, it was calculated that the k_{cat}

in the presence and absence of α_2 -M was 4.0 s^{-1} and 0.45 s^{-1} respectively, whereas the K_m was $1.82 \times 10^{-3}\text{ M}$ both in the presence and absence of α_2 -M. The fact that there was only a ninefold enhancement of the k_{cat} of elastase in the presence of α_2 -M compared to the 15-fold increase in the activity ratio (Fig. 1) may be due in part to the fact that in the latter case activity was based on end-point measurements at 37°C whereas the kinetic analysis was based on measurements of initial rates at 25°C .

Table 1 summarises data pertaining to the effect of α_2 -M on the activity of leukocyte elastase towards various other synthetic substrates. In addition, a comparison has been made with porcine pancreatic elastase (Worthington) in the case of Suc-(Ala)₃-Nan. The greatest degree of activation of the leukocytic elastase by human α_2 -M was observed with Suc-(Ala)₃-Nan as the substrate. Replacing the acidic succinyl group with an acetyl or a butoxy-carbonyl group produced a lesser degree of activation, and the substitution of the aromatic component of the ester linkage by a methyl group caused little, if any, activation. Of further interest is the fact that the activity of porcine pancreatic elastase towards Suc-(Ala)₃-Nan was actually inhibited by human α_2 -M.

Experiments were performed to ascertain whether this phenomenon of activation could be demonstrated with sera obtained

Fig. 1 Activation of human leukocytic elastase towards Suc-(Ala)₃-Nan by human α_2 -M. Reaction mixtures contained 100 μl of enzyme solution ($18\text{ }\mu\text{g ml}^{-1}$) with various levels (0–100 μl) of α_2 -M solution (1 mg ml^{-1}) in a final volume of 1.2 ml 0.2 M Tris-HCl–0.5 M NaCl, pH 8. After pre-incubation for 15 min at 37°C , 150 μl of substrate solution (0.01 M Sac-(Ala)₃-Nan; ref. 13) were added and the incubation contained for a further 20 min at the same temperature. Reaction was terminated by adding 150 μl glacial acetic acid, and the absorbance read at 410 nm. Insert is plot of the increase in absorbance (absorbance of enzyme with α_2 -M minus absorbance of enzyme without α_2 -M) as a function of the quantity of α_2 -M added. In the larger figure, the activity ratio (ratio of absorbance of enzyme with α_2 -M to absorbance of enzyme without α_2 -M) is plotted as a function of the ratio (by weight) of α_2 -M to enzyme.

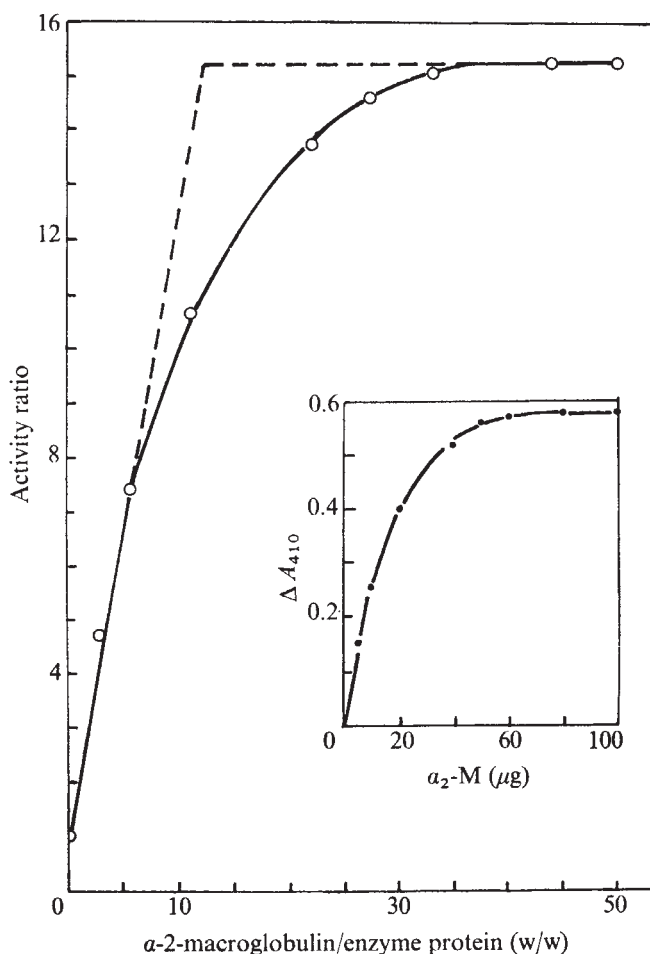


Table 1 Effect of human α_2 -M on the activity of elastases towards various synthetic substrates

Substrate*	Source of elastase	Specific activity †		Ratio + α_2 -M / - α_2 -M
		- α_2 -M	+ α_2 -M	
Suc-(Ala) ₃ -Nan	Leukocytes (human)	0.3	4.6	15.3
Suc-(Ala) ₃ -Nan	Pancreatic (porcine)	1.8	0.8	0.4
Ac-(Ala) ₃ -Nan	Leukocytes (human)	1.2	4.2	3.5
Boc-Ala-ONp	Leukocytes (human)	3.4	7.3	2.2
Ac-(Ala) ₃ -OMe	Leukocytes (human)	56.7	59.7	1.1

*Suc-(Ala)₃-Nan, *N*-succinyl-L-alanyl-L-alanyl-L-alanine-4-nitroanilide (assay in ref. 13); Ac-(Ala)₃-Nan, *N*-acetyl-L-alanyl-L-alanyl-L-alanine-4-nitroanilide (assay ref. 14); Boc-Ala-ONp, *N*-*t*-outoxycarbonyl-L-alanine-4-nitrophenylester (assay ref. 15); Ac-(Ala)₃-OMe, *N*-acetyl-L-alanyl-L-alanyl-L-alanine methyl ester (assay ref. 16).

†Units of activity per mg enzyme where one unit of activity is equivalent to the hydrolysis of 1 μ mol of substrate per min under the conditions specified for each assay.

from normal (phenotype *MM*) and α_1 -AT-deficient (phenotype *ZZ*) individuals. Data were obtained with sera from two patients who had been diagnosed as being α_1 -AT-deficient homozygotes (phenotype *ZZ*) and with sera from two normal individuals (phenotype *MM*) (Table 2). Both the normal and deficient sera completely inhibited the activity of the leukocyte and pancreatic elastases toward elastin. When the synthetic substrates were tested, the residual activity of both elastases was consistently higher in the case of the *ZZ* sera. Especially noteworthy, however, is the fact that there was a fourfold increase in the activity of the leukocyte elastase when tested against Suc-(Ala)₃-Nan in the presence of the *ZZ* sera. This observation can be readily explained by the fact that the predominant protease inhibitor in *ZZ* sera is α_2 -M rather than α_1 -AT, a situation which, on the basis of our previous experiments, would be expected to augment the activity of the leukocytic elastase towards Suc-(Ala)₃-Nan.

α_2 -M is known to interact with a wide variety of proteases to form complexes which are generally inactive towards large molecular weight substrates but which exhibit little or no loss in activity towards low molecular weight substrates¹⁷. Although there have been occasional reports of an enhancement of the activity of trypsin (bovine)¹⁸, chymotrypsin (bovine)¹⁹, and elastase (porcine)²⁰ by α_2 -M (human), the degree of activation has usually been quite small (10–50%) compared to that which we observed with our system (900–1,500%). We attribute this difference to two factors; first, both the enzyme and α_2 -M which we used were derived from the same species of animal; and second, the substrate used had an aromatic moiety as part of the ester or amide linkage and had an acidic group blocking the terminal amino group of the substrate. It is interesting to note that Nakamura *et al.*²¹ have also reported a two to threefold activation of bovine chymotrypsin by bovine α_2 -M on substrates possessing these same attributes. In agreement with the data of Nakamura *et al.*²¹ we also found that maximum activation of leukocyte elastase towards a synthetic substrate was produced when two molecules of the enzyme were complexed by one molecule of human α_2 -M. Ohlsson and Laurell⁸ have reported a similar ratio for the inactivation of

leukocyte elastase by human α_2 -M with elastin as the substrate.

At the moment we are unable to explain this phenomenon of activation, but any explanation that is offered should take into account the hypothesis that the complexation of proteases with α_2 -M is probably a two-step process involving an initial cleavage of a critical peptide bond followed by a conformational change which serves to entrap the enzyme¹⁷. Macromolecular substrates are thus prevented from reaching the enzyme, whereas small synthetic substrates are not so restricted. Particularly puzzling is the fact that the K_m , which reflects the affinity of the enzyme for the substrate, Suc-(Ala)₃-Nan, is not affected by α_2 -M despite a marked enhancement in the catalytic efficiency of the enzyme as measured by k_{cat} . Since the latter effect seems to depend on the presence of an aromatic and an acidic group on the substrate molecule, it may be that the conformational change in α_2 -M which serves to entrap the enzyme uncovers a hydrophobic cavity which, in conjunction with a positively charged group, orients the substrate in a position which is more favourable for acylation or deacylation by the enzyme. Jacquot-Armand and Krebs²² have in fact shown that α_2 -M which has interacted with trypsin undergoes a conformational change which exposes an apolar region in the α_2 -M molecule. Furthermore, it seems that the most striking effects are produced when the enzyme and α_2 -M are derived from the same species of animal—the reason for this remains obscure.

Whether this phenomenon of activation is of any physiological significance is open to conjecture. One observation which may be pertinent in this respect is the reference by Meyer *et al.*²³ to unpublished results showing that pancreatic elastase (porcine) was capable of digesting chemically solubilised elastin even when complexed with α_2 -M. If the leukocytic elastase were to behave in a comparable fashion, then its complexation with α_2 -M might actually serve to augment its ability to degrade tropoelastin secreted into the extracellular space prior to the formation of cross-linkages found in elastin²⁴. In normal individuals this destructive effect on the synthesis of elastin could be held in check by adequate levels of α_1 -AT. On the other hand, as suggested by our *in vitro* experiments with *ZZ* sera, in individuals who are deficient in α_1 -

Table 2 Effect of *MM* and *ZZ* sera on the activity of leukocyte and pancreatic elastases on various substrates *

Substrate†	Source of elastase	Phenotype‡				N.G.
		M.G.	<i>MM</i> P.L.	<i>ZZ</i> J.H.	<i>ZZ</i> J.H.	
Elastin-rhodamine	Leukocytes (human)	0	0	0	0	0
	Pancreatic (porcine)	0	0	0	0	0
Suc-(Ala) ₃ -Nan	Leukocytes (human)	55	70	400	400	400
	Pancreatic (porcine)	8	13	40	40	45
Boc-Ala-ONp	Leukocytes (human)	10	—	50	48	48
	Pancreatic (porcine)	15	11	55	49	49

*Activities are expressed as % of controls without serum.

†Activity towards elastin-rhodamine was measured according to Rinderknecht *et al.*²⁵ using levels of 1.4 and 12.5 μ g of porcine and leukocyte elastase respectively in each assay mixture. Assays on synthetic substrates were performed as described in Table 1 using levels of 1.4 and 1.33 μ g of porcine and leukocyte elastase respectively in each assay system. The level of serum employed in these assays was 1.0 μ l serum per μ g enzyme in case of *MM* sera and 4.5 μ l serum per μ g enzyme for *ZZ* sera.

‡The trypsin inhibitor capacity of the serum of each individual was assayed on *N*-benzoyl-DL-arginine-*p*-nitroanilide²⁶, and the following values, expressed as % of a standard reference serum (supplied by Dr John A. Pierce) were obtained for the four individuals: M.G., 119; P.L., 100; J.H., 32; N.G., 25.

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*Department of Biochemistry,
College of Biological Sciences,
University of Minnesota,
St Paul, Minnesota 55108*

*Department of Medicine,
School of Medicine,
New York University Medical Center,
New York, New York 10016*

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INCREASED susceptibility to infectious disease is observed in iron-overloaded states such as β -thalassaemia major and in haemolytic states such as sickle cell disease or Bartonellosis^{1,2}. The virulence of *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium* and other pathogens can be increased by administration of iron compounds to the host, and transferrin-induced inhibition of bacterial growth can be reversed by saturating transferrin³⁻⁶. Plasma and secretory iron-binding proteins such as transferrin and lactoferrin may function in host defence by limiting the availability of iron to invading pathogens⁷. Many micro-organisms synthesise iron-binding compounds when little iron is available⁸; these compounds may compete with host iron-binding mechanisms. We report here the effects of iron-chelating drugs on this competition for iron in *S. typhimurium* infection in normal and iron-overloaded mice. Desferrioxamine (DF), a hydroxamic acid, and 2,3-dihydroxybenzoic acid (2,3-DHB), a phenolic iron chelator, represent two classes of iron-chelating compounds which are being tested in chelation therapy for iron overload. Our data provide evidence that DF increases iron availability to wild type *S. typhimurium*, promoting bacterial growth and thus enhancing virulence.

Male Swiss-Webster mice were iron overloaded by intraperitoneal injections of 0.25 ml of human blood heated to 50 °C for 5 min. Injections were given once a day, five

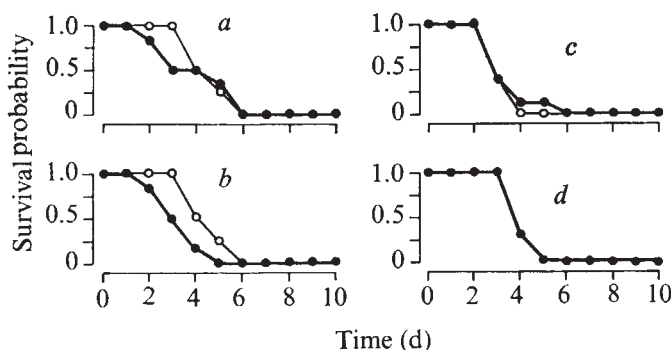
Wild type *S. typhimurium* was obtained from a clinical microbial laboratory and found to grow normally on low-iron medium. A mutant of *S. typhimurium* (TA2701) blocked in the synthesis of enterobactin, its own natural iron chelator, was unable to grow on low-iron media in the presence of DF but would grow in response to 2,3-DHB (refs 10, 11). After passage through six generations of mice to increase its virulence, the mutant retained its growth characteristics on low-iron medium. Organisms for injection were cultured overnight in nutrient broth, washed three times and diluted in sterile saline.

Iron-overloaded and normal mice received an intraperitoneal injection of either the wild type or mutant *S. typhimurium* and were divided into three groups of six. One group received DF, one received 2,3-DHB and the third received no drug. Both drugs were injected in doses of 25 mg kg⁻¹ every 6 h, beginning 24 h before infection and continuing throughout the experiment. Uninfected groups of six iron-overloaded or normal mice received DF or 2,3-DHB in the same doses. The number of survivors from each group was recorded at 6-h intervals.

Cultured post mortem liver specimens yielded organisms with the same growth characteristics on low-iron medium as the infecting organism. This confirms that the pathogen injected was the cause of death. All uninfected mice treated with 2,3-DHB or DF survived.

Figure 1 shows the effects of iron overloading on the survival probability of animals not receiving an iron-chelating drug. In the groups infected with wild type *S. typhimurium*, iron overloading caused a significant reduction in survival probability. Similarly, in the groups treated with DF and 2.3-DHB (not shown), iron overloading increased virulence in wild type infection, the effect being

Fig. 1 Survival probabilities against time of normal (○) and iron-overloaded (●) mice infected with *S. typhimurium*. *a*, Wild type, 10^3 bacteria, $P < 0.02$; *b*, wild type, 10^6 bacteria, $P < 0.02$; *c*, mutant TA2701, 10^4 bacteria, not significant; *d*, mutant TA2701, 10^6 bacteria, not significant. Probabilities were determined by the Stromgren method of life-table analysis¹². Differences in the probability plots were compared for significance at the maximum deviation using a two-sided hypothesis¹³.



most pronounced with DF. In untreated mice infected with the mutant (Fig. 1b), iron overloading had no effect on survival probabilities. Similarly, iron overloading produced no reduction in survival in DF-treated animals infected with the mutant. But in animals infected with the mutant and treated with 2,3-DHB, survival was reduced by iron overloading (Fig. 2).

Figure 3 shows that in wild type infection, survival of both normal and iron-overloaded animals was reduced significantly by DF. This effect was absent or reversed in animals infected with the mutant. The reversal of the DF effect—prolongation of survival—was seen in both normal and iron-overloaded animals given the lower infecting dose.

Wild type *S. typhimurium* which grow in low-iron media can do so, at least in part, because they synthesise an iron chelator such as enterobactin¹⁴. *In vivo*, if iron overloading produces an increase in host iron available to the pathogen, wild type growth rate and thus virulence would be expected to increase. This expectation was fulfilled in control and drug-treated animals which were iron overloaded and infected with the wild type *Salmonella*. If the ability to synthesise an iron chelator is absent, as in mutant TA2701, increased availability of iron in the host should have no effect on virulence. This is confirmed by Fig. 1b. The only mutant-infected animals to show reduced survival with iron overloading were those treated with 2,3-DHB. This was anticipated by the *in vitro* growth characteristics of TA2701, which can utilise 2,3-DHB as an iron chelator.

DF has been shown to increase the growth rate of *S. typhimurium* in defined medium¹⁰, and Fig. 3 confirms this effect *in vivo*. DF seems to increase the availability of iron to multiplying wild type *Salmonella* and thus to reduce the survival of the host. This effect is augmented by iron overloading. On the other hand, treatment of the mutant-infected animals with DF produced no significant increase in virulence. At the lower levels of infection in both normal and iron-overloaded animals, DF significantly prolonged survival. These results support the *in vitro* observation that the mutant cannot utilise ferrioxamine. Thus by sequestering iron and making it unavailable to the mutant, DF retards its growth and decreases its virulence.

These experiments provide evidence that DF, which is used to treat iron overload, may increase the virulence of *S. typhimurium* by increasing the iron available to the bacteria and thereby promoting growth. The results also suggest that an iron chelator not utilisable by micro-organisms decreases the iron available for bacterial growth

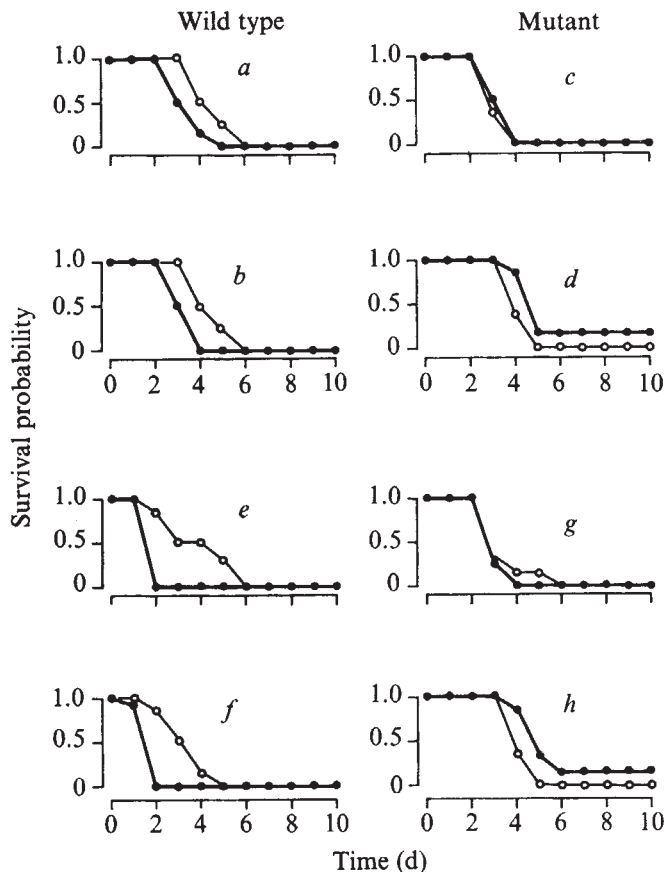
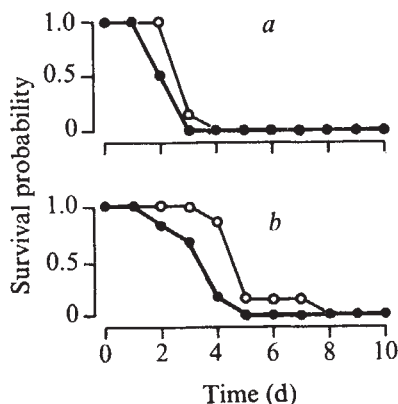


Fig. 3 The effect of DF (Ciba-Geigy) on the survival probabilities of normal (a-d) and iron-overloaded (e-h) mice infected with *S. typhimurium*. a, 10^5 bacteria, $P < 0.02$; b, 10^3 bacteria, $P < 0.02$; c, 10^6 bacteria, not significant; d, 10^4 bacteria, $P < 0.05$, e, 10^5 bacteria, $P < 0.001$; f, 10^3 bacteria, $P < 0.001$; g, 10^6 bacteria, not significant; h, 10^4 bacteria, $P < 0.05$. ○, Control (no drug); ●, treated with DF.

Fig. 2 Survival probabilities of normal (○) and iron-overloaded (●) mice treated with 2,3-DHB and infected with mutant TA2701. 2,3-DHB was synthesised in our laboratory⁹. a, 10^6 bacteria, $P < 0.02$; b, 10^4 bacteria, $P < 0.01$.



and thus decreases the virulence of invading pathogens.

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ROBERT L. JONES
CHARLES M. PETERSON
ROBERT W. GRADY
TURKAN KUMBARACI
ANTHONY CERAMI

Rockefeller University,
New York, New York 10021

JOSEPH H. GRAZIANO

New York Hospital,
Cornell Medical Center,
New York, New York 10021

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GABA receptor binding with ³H(+) bicuculline-methiodide in rat CNS

NEUROTRANSMITTER receptors might be identified biochemically in binding studies with synaptic membrane preparations using labelled receptor agonists or antagonists as ligands¹. In attempts to identify the GABA receptor ³H-GABA has been used as ligand. ³H-GABA, however, also binds to glial and neuronal GABA uptake sites and to enzymes metabolising GABA. Thus, Na⁺-dependent ³H-GABA-binding^{2,3}, in contrast to Na⁺-independent ³H-GABA-binding is possibly not related to the GABA receptor⁴. In the present investigation the GABA antagonist ³H(+)bicuculline-methiodide (³H(+)BM) was used as ligand, which in contrast to bicuculline, is chemically sufficiently stable⁵. (+)Bicuculline and its N-methyl-derivative antagonise reversibly and rather selectively the inhibitory synaptic action of GABA in the central nervous system (CNS) (refs 6, 7) without affecting the uptake or release of GABA or enzymes metabolising GABA (refs 8, 9). Thus ³H(+)BM might be more suitable for GABA receptor binding studies than GABA itself.

The characteristics of specific ³H(+)BM binding in the present study are compatible with the properties of the GABA receptor expected from neurophysiological data. Specific ³H(+)BM-binding was saturable, stereospecific and maximally enriched in the synaptic membrane fraction. Among a variety of compounds tested only GABA, GABA agonists and GABA antagonists were potent competitors for ³H(+)BM specific binding sites.

Routine binding assays were performed by incubating crude cerebellar synaptic membranes with 5 nM ³H(+)BM in the absence and presence of exogenous GABA. 'Specific binding' of ³H(+)BM to the membranes was obtained by subtracting from the radioactivity bound in the absence of GABA ('total binding') the amount not displaceable by high concentrations of GABA (50 μ M; 'non-specific binding').

Among the different subcellular fractions of cerebellum

tested, the highest specific ³H(+)BM binding was found in the crude synaptic membrane fraction (Table 1). The specific activity (d.p.m. per mg protein) of ³H(+)BM specifically bound in this fraction was about 2.5 times that of the whole homogenate and similar to that of the P₂-fraction containing intact synaptosomes.

Specific ³H(+)BM binding to the cerebellar synaptic membrane fraction was saturable, half-maximal binding occurring at 380 $\pm$ 20 nM. By contrast, non-specific binding, represented by the radioactivity bound in the presence of 50 μ M GABA, was not saturable and increased linearly with increasing ³H(+)BM concentration. Similarly, displacement of ³H(+)BM by non-radioactive (+)BM was half-maximal at 218 $\pm$ 21 nM (Table 2, Fig. 2), correlating well with the value obtained in the saturation experiments using ³H(+)BM alone.

The stereospecificity of ³H(+)BM binding is shown by the finding that the neurophysiologically inactive isomers (–)bicuculline and (–)BM (ref. 10) had only about 1/800 and 1/400 the affinity of the respective (+) isomers for the ³H(+)BM binding sites, as indicated by their K_i values (Table 2, Fig. 1). Furthermore, when ³H(–)BM was used as ligand (5 nM), no displacement with 50 μ M non-radioactive (+)BM, GABA, muscimol, 3-aminopropane-sulphonic acid or (–)BM was observed, indicating that the neurophysiologically inactive isomer (–)BM did not bind to the (+)BM binding sites at the ligand concentration used. In addition, since the excitatory action of (+)BM found *in vivo* is not stereospecific¹⁰, this action does not seem to be reflected in the *in vitro* ³H(+)BM binding studies presented here.

The high affinity of (+)bicuculline (K_i = 68 nM) for the (+)BM binding sites (Table 2, Fig. 1) corresponds to its high potency as a GABA antagonist *in vivo*; (+)bicuculline is about 60 times less potent in competing for Na⁺-independent ³H-GABA binding sites (rat whole brain⁴) and even 1,000 times (rat cerebellar cortex³) and 70,000 times (crayfish muscle²) less potent in competing for Na⁺-dependent ³H-GABA binding sites.

The findings that (+)BM is more potent than (+)bicuculline as a GABA antagonist microelectrophoretically⁷ and as a convulsant¹¹ are difficult to explain on the basis of their potencies in competing for ³H(+)BM binding sites (Table 2, Fig. 1). However, the high lipid solubility of bicuculline might influence its concentration at the receptor or binding sites to a varying degree under different experimental conditions. In addition, there might be a

Table 1 Subcellular distribution of specifically bound ³H(+)BM in rat cerebellum

Fraction	Specifically bound ³ H(+)BM (d.p.m. per mg protein) (as % of total binding)		Specifically bound ³ H(+)BM per entire fraction (d.p.m.)
Whole homogenate	471 $\pm$ 34	16.7 $\pm$ 1.0	225,200 $\pm$ 10,400
Crude nuclear pellet (P ₁)	318 $\pm$ 97	13.5 $\pm$ 3.3	85,600 $\pm$ 25,400
Crude mitochondrial pellet (P ₂)	1,082 $\pm$ 17	28.7 $\pm$ 0.5	128,100 $\pm$ 6,500
Crude microsomal pellet (P ₃)	204 $\pm$ 14	8.9 $\pm$ 0.8	11,390 $\pm$ 1,170
P ₂ subfractions after osmotic shock:			
Mitochondria-myelin pellet	422 $\pm$ 7	14.9 $\pm$ 0.2	7,900 $\pm$ 1,050
Crude synaptic membrane pellet	1,044 $\pm$ 34	28.7 $\pm$ 0.7	116,600 $\pm$ 5,100

The subcellular fractions of cerebellum (male SPF rats, 150–200 g) were prepared according to Zukin *et al.*⁵. The binding assay was performed in triplicate as described in Fig. 1 (5 nM ³H(+)BM and 2 mg protein per assay in the presence or absence of 50 μ M GABA). 'Total binding' refers to the radioactivity bound in the absence of GABA, 'specifically bound' to that displaceable by GABA. The values are the means $\pm$ s.e.m. from three experiments.

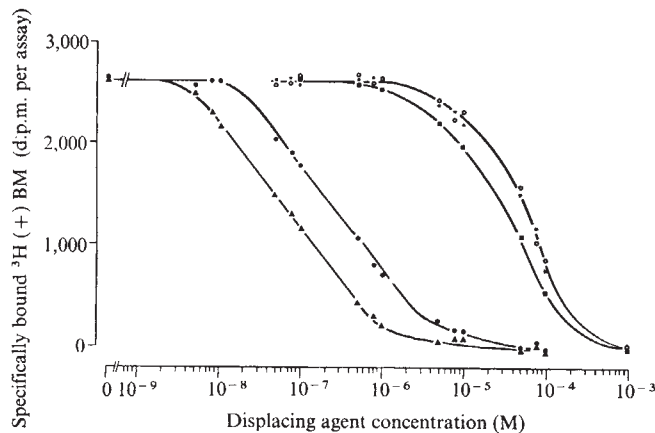


Fig. 1 Competition for the $^3\text{H}(+)\text{BM}$ specific binding sites by (+) bicuculline (▲); (—) bicuculline (*) (●); (+) bicuculline methiodide (○); (—) bicuculline methiodide (◊); (±) laudanosine (■). Increasing concentrations of the displacing agent were added to tubes containing in a 2 ml volume cerebellar synaptic membranes (2 mg protein), 5 nM $^3\text{H}(+)\text{BM}$, 50 mM Tris-HCl buffer pH 7.4 and 50 mM NaSCN. After incubation at 25 °C for 15 min—at which time equilibrium had been reached—and centrifugation at 48,000 g for 10 min, the pellet was rinsed by superfusion with 5 ml and then 10 ml of ice-cold 50 mM Tris-HCl buffer pH 7.4. After dissolving the pellet in 2 ml Protosol, scintillant was added and the radioactivity counted (counting efficiency 38%). The points are the means of triplicate determinations of a typical experiment with standard errors of the mean ranging from 0.1 to 2.0%. The corresponding K_i values are given in Table 2. $^3\text{H}(+)\text{BM}$ and $^3\text{H}(-)\text{BM}$ (radiochemically pure > 99%; specific activity 8.0 and 13.0 Ci per mmol respectively) were prepared by methylation¹⁵ of (+) and (—) bicuculline with ^3H -labelled methyl iodide synthesised according to Schwob and Würsch¹⁶. (—) Bicuculline was synthesised according to Teitel *et al.*¹⁷. Crude synaptic membrane fractions were prepared according to Enna and Snyder⁴ and stored frozen at -30 °C for 1–5 d before use. Fresh and frozen membranes gave similar results. There was no degradation of bound or free $^3\text{H}(+)\text{BM}$ during the incubation.

contribution from the excitatory effect of (+)BM *in vivo* or a regional or species difference in the potency of the two compounds.

In agreement with the neurophysiological potency⁸, the GABA agonist muscimol was more, imidazole acetic acid less potent in competing for $^3\text{H}(+)\text{BM}$ binding sites than GABA itself, while 3-aminopropanesulphonic acid despite

Fig. 2 Competition for the $^3\text{H}(+)\text{BM}$ specific binding sites by GABA and GABA agonists. The binding assay was performed as described in Fig. 1 (5 nM $^3\text{H}(+)\text{BM}$; 2 mg cerebellar synaptic membrane protein per assay). Points are means of triplicate determinations in a typical experiment with s.e.m. values of 0.1–2.5%. The corresponding K_i -values are given in Table 2. Muscimol (▲); 3-aminopropanesulphonic acid (●); GABA (*) (◊); imidazole acetic acid (■).

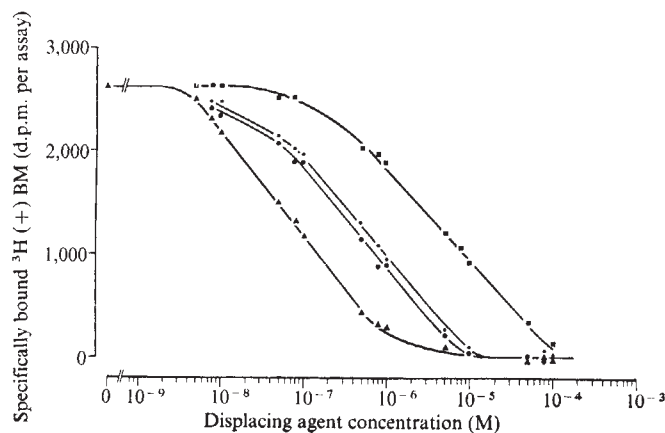


Table 2 Inhibition of specific $^3\text{H}(+)\text{BM}$ -binding to synaptic membranes of rat cerebellum by various compounds

Compound	K_i (nM)
GABA antagonists	
(+) Bicuculline	68.2 ± 2.5
(+) Bicuculline-methiodide	218 ± 21
GABA agonists, amino acids and related compounds	
Muscimol	58.1 ± 6.8
GABA	420 ± 55
3-Aminopropanesulphonic acid	490 ± 70
Imidazole acetic acid	3,830 ± 320
β-Alanine	58,700 ± 6,300
Glutamate	n.i.
Aspartate	n.i.
α-Alanine	n.i.
Glycine	n.i.
Proline	n.i.
Taurine	n.i.
Nipecotic acid	n.i.
Aminooxyacetic acid	n.i.
Isoniazid	n.i.
Other compounds	
Strychnine	3,500 ± 360
(±) Laudanosine	33,700 ± 1,600
(—) Bicuculline-methiodide	52,800 ± 5,200
(—) Bicuculline	59,300 ± 3,500

'No inhibition' (n.i.) refers to concentrations up to 10^{-4} M. Further compounds without inhibitory effect at least up to 10^{-5} M include 2,4-diaminobutyrate, morphine, nialamide, kainic acid, chlordiazepoxide, diazepam, pentobarbital, baclofen, oxotremorine, physostigmine and Ro 4-1284, a water-soluble benzoquinolizine derivative with reserpine-like action¹⁸. Haloperidol and chlorpromazine displaced 18% of the specifically bound $^3\text{H}(+)\text{BM}$ at 10^{-5} M. Inhibition of $^3\text{H}(+)\text{BM}$ -binding (5 nM; 2 mg cerebellar membrane protein per assay) was measured at six to 15 concentrations of each compound in triplicate as described in Fig. 1. The 50% inhibitory concentration (IC_{50}) was calculated by log logit analysis and converted to K_i according to the equation $K_i = \text{IC}_{50}/(1 + C/K_D)$, where C is the concentration of radioactive ligand and K_D its dissociation constant. The values are the means ± s.e.m. of three experiments. For some compounds typical inhibition curves are shown in Figs 1 and 2.

its higher neurophysiological potency¹² was similar to GABA in its competing potency (Table 2, Fig. 2). As drug potencies can differ in different species and/or brain areas, however (as shown for muscimol and bicuculline⁸), a more quantitative correlation has to await binding experiments and neurophysiological studies to be performed in the same species and brain region.

It should be noted that (±)laudanosine, a neurophysiologically inactive analogue of bicuculline^{6,7}, is also inactive as inhibitor of specific $^3\text{H}(+)\text{BM}$ binding (Table 2, Fig. 1). Strychnine, with an affinity constant for the glycine receptor of 2 nM (ref. 13), required concentrations 1,700 times higher to reduce specific $^3\text{H}(+)\text{BM}$ binding by 50%.

In studies of the distribution of specifically bound $^3\text{H}(+)\text{BM}$ in different brain regions the highest levels were found in cerebral cortex (82.7 ± 1.4 fmol per mg protein) and cerebellum (67.6 ± 1.2), followed by hippocampus (57.1 ± 1.5), midbrain (54.2 ± 2.5), hypothalamus (49.4 ± 2.1) and striatum (39.2 ± 1.9). The lowest levels were found in medulla oblongata-pons (16.1 ± 1.7) and spinal cord (8.7 ± 2.0). These regional differences do not correlate well with the regional levels of endogenous GABA in rat CNS¹⁴. In other receptor systems, however, there are also discrepancies between receptor densities and endogenous transmitter levels. Presumably, the surface area and receptor density of the postsynaptic membrane vary independently of the endogenous transmitter levels in different areas of brain¹. The regional distribution of specific $^3\text{H}(+)\text{BM}$ -binding is largely similar to that of Na-independent ^3H -GABA-binding with the cerebellum as main exception which shows twice the GABA-binding of cerebral cortex⁴.

The GABA agonists tested so far in Na⁺-independent

^3H -GABA-binding* and in $^3\text{H}(+)\text{BM}$ -binding (GABA, 3-aminopropanesulphonic acid, imidazole acetic acid) show a higher affinity for ^3H -GABA binding sites than for $^3\text{H}(+)\text{BM}$ binding sites. The inverse relationship holds for (+)bicuculline, the only GABA antagonist compared so far. This finding may be explained by a receptor model in which agonists and antagonists bind to two different receptor sites possibly with a mutual negative cooperative interaction. Binding of an agonist might reduce the binding affinity of an antagonist and vice versa. But as the rank orders of regional ^3H -GABA-binding and $^3\text{H}(+)\text{BM}$ -binding are not identical, we cannot exclude the possibility that ^3H -GABA and $^3\text{H}(+)\text{BM}$ label different types of GABA receptors at least in some rat brain areas.

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H. MÖHLER
T. OKADA*

Pharmaceutical Research Department,
F. Hoffmann-La Roche & Co. Ltd,
4002 Basle, Switzerland

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*Permanent address: Biochemistry Department, Nippon-Roche Research Centre, 200 Kajiura, Kamakura-City, Japan.

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Direct detection of depolarisation-induced release of ATP from a synaptosomal preparation

SEVERAL groups have shown that ATP is released from neural tissues during depolarisation, where it or its metabolites might function as neurotransmitters or neuromodulators¹. They measured radiolabelled adenosine derivatives in perfusates following depolarisation of their preparations. Recently, Israel *et al.*² described a method for continuously and directly detecting the release of ATP from the electric organ of *Torpedo marmorata* after stimulation of its motor nerve. I have used a modification of this technique to study the depolarisation-induced release of ATP from a purified synaptosomal fraction prepared from rat brain. I report here that depolarisation of synaptosomes elicits a release of ATP which can be directly and continuously detected by this technique.

Synaptosomes were incubated in a Krebs–Henseleit bicarbonate medium containing firefly luciferin–luciferase and depolarised by elevating the extracellular K^+ or by adding veratridine. Veratridine depolarises excitable tissues, including synaptosomes, by selectively opening Na^+ channels^{3–6}. Released ATP was detected by measuring the light emitted with a photomultiplier. Supplementing the luciferin⁷ greatly enhanced the sensitivity of detection of ATP. Preliminary experiments showed that the assay system was specific for ATP, in that similar concentrations of adenosine, AMP or ADP did not produce chemiluminescence.

Depolarisation of synaptosomes caused a release of ATP

which could be directly and continuously monitored. Elevating the extracellular KCl concentration by 23 mM caused a rapid release of ATP from synaptosomes, indicated by an increased emission of light (Fig. 1a). This effect was probably not due to the increased tonicity of the incubation medium, since increasing the extracellular concentration of NaCl by 23 mM caused a fall in the background chemiluminescence. Choline chloride and LiCl produced similar decreases in chemiluminescence (not shown). The response to elevated extracellular K^+ was not prevented by the specific Na^+ channel blocker, tetrodotoxin^{9–10} (Fig. 1b), showing that this effect was not mediated by the opening of Na^+ channels in the synaptosomal membranes. This is not surprising, since one would expect elevated extracellular K^+ to depolarise the synaptosomes directly without altering the Na^+ and K^+ permeabilities^{11,12}.

The depolarisation-induced release of a number of putative neurotransmitters from brain preparations has been

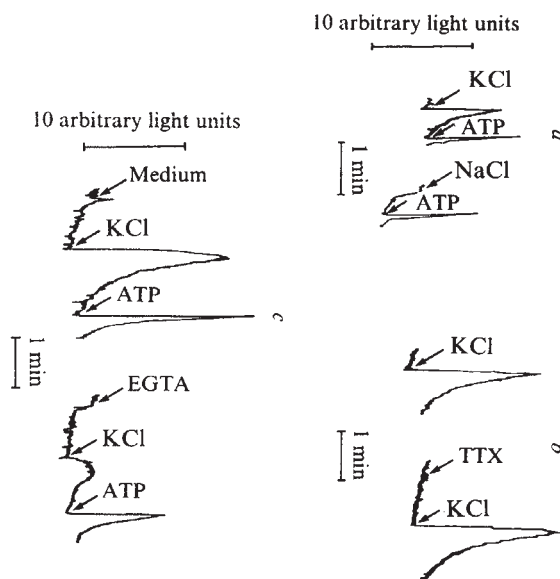


Fig. 1 K^+ -induced release of ATP from synaptosomes. Synaptosomes were prepared from the whole brains of four adult male Wistar rats (200–250 g) and isolated on sucrose density gradients as described previously⁸. They were finally suspended to a final volume of 8 ml with Krebs–Henseleit bicarbonate medium containing 111 mM NaCl , 26.2 mM NaHCO_3 , 1.2 mM NaH_2PO_4 , 4.7 mM KCl , 1.8 mM CaCl_2 , 1.2 mM MgCl_2 , 11 mM glucose and gassed periodically with 95% O_2 : 5% CO_2 to maintain a pH of 7.4. The suspension was preincubated at 37 °C for 30 min and a 0.5 ml portion placed in a 6 × 50 mm cylindrical cuvette; 20 μl of fortified luciferin–luciferase enzyme preparation were added. The enzyme preparation was prepared daily by adding 0.2 ml synthetic D-luciferin (5 mg per ml H_2O , Sigma; stored at −20 °C) to 0.2 ml of crude firefly lantern extract (50 mg Sigma FLE-50 in 2 ml H_2O , prepared daily). The cuvette was placed in the reaction chamber of a ‘Chem-Glow’ chemiluminometer (American Instrument Co.) and let stand until a reasonably constant level of chemiluminescence was attained, usually 1–2 min. Even without gassing, the pH of the suspension remained at 7.5–7.6 throughout the incubation. *a*, KCl was increased by 23 mM by injecting 10 μl of a 1.25 M KCl solution into the cuvette with a Hamilton syringe. After the chemiluminescence had returned to its resting level, 5 μl of a 10^{-7} M ATP solution in incubation medium were injected into the cuvette to give a final ATP concentration of 9.4×10^{-10} M. The experiment was repeated using NaCl instead of KCl . *b*, Injecting 10 μl of 2.2×10^{-4} M tetrodotoxin (TTX) dissolved in incubation medium into the cuvette (Calbiochem, final concentration 4×10^{-7} M) did not prevent the release of ATP by elevated extracellular K^+ (increased by 47 mM). *c*, To chelate Ca^{2+} , 10 μl of 250 mM EGTA (pH 7.4 with NaOH) were injected into the cuvette (final concentration of 4.7 mM EGTA). Removal of Ca^{2+} by EGTA reduced the maximal response to added ATP to about 57% of the control value but reduced the maximal response to increased extracellular KCl to about 21% of the control value.

shown to be Ca^{2+} -dependent¹³⁻¹⁶. In the present study, the presence of 4.7 mM EGTA to chelate Ca^{2+} seemed to reduce the release of ATP by elevated extracellular K^+ (Fig. 1c), but EGTA also reduced the sensitivity of the luciferin-luciferase system to added ATP. Closer examination shows, however, that EGTA reduced the maximum K^+ response to about 21% of the control value, whereas EGTA only reduced the sensitivity of the system to added ATP to about 57% of the control. It therefore seems that the release of ATP from synaptosomes by elevated extracellular K^+ is at least in part Ca^{2+} -dependent.

Injection of veratridine into a synaptosomal suspension caused a rapid release of ATP which could be prevented by the prior administration of tetrodotoxin (Fig. 2). Tetrodotoxin added at the peak of the veratridine response caused an abrupt fall in the observed release of ATP. These findings indicate that the release of ATP by veratridine, in contrast to that caused by elevated K^+ (see Fig. 1), was mediated by the opening of Na^+ channels. Surprisingly, veratridine produced a more prolonged release of ATP than did elevated K^+ . The nature of this prolonged release of ATP is currently under investigation.

This study clearly demonstrates a depolarisation-induced and possibly Ca^{2+} -dependent release of ATP from synaptosomal preparations. As to the origin of the ATP

released, it seems most likely that presynaptic nerve-terminals are involved, although it is also possible that glial contaminants of the synaptosomal preparation¹⁷ might respond to depolarising influences by releasing ATP. Elevated extracellular K^+ has been reported to release γ -aminobutyric acid from glial cells in dorsal root ganglia by a Ca^{2+} -dependent mechanism¹⁸. On the other hand, the veratridine-induced release of ATP from synaptosomal preparations indicates an involvement of Na^+ channels, which glial elements may not possess.

Whether or not the ATP is released in its own right or in conjunction with the release of neurotransmitters¹³⁻¹⁶ is not known. In either case, it does not seem reasonable that such an energetically valuable molecule as ATP would be released and hydrolysed simply to provide a source of adenosine to promote the post-synaptic synthesis of cyclic-3', 5' AMP (ref. 1). Perhaps the extracellular hydrolysis of released ATP provides energy for some functionally important process in the synaptic region.

Numerous other questions arise as to the nature, distribution and role of the ATP release process in brain. This new method for directly studying the depolarisation-induced release of ATP from synaptosomes may assist in answering these questions.

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THOMAS D. WHITE

Department of Pharmacology,
Dalhousie University,
Halifax, Nova Scotia,
Canada B3H 4H7

Received 11 January; accepted 25 February 1977.

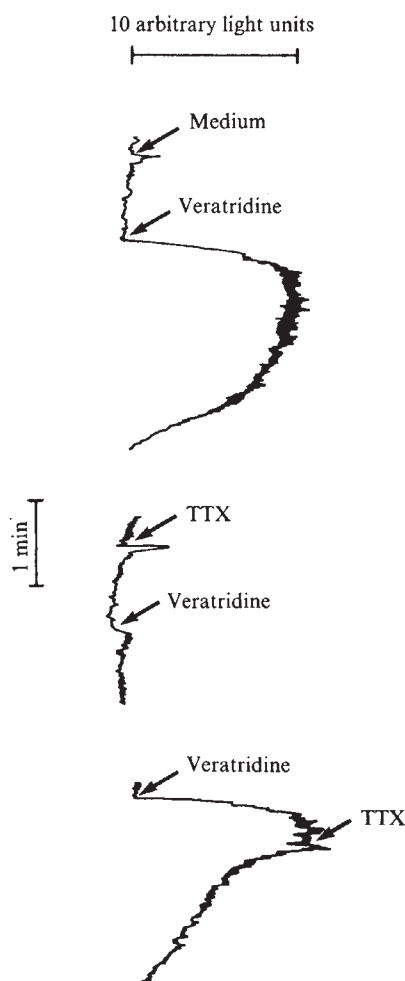


Fig. 2 Effect of veratridine on the release of ATP from synaptosomes. Synaptosomes were prepared and incubated as for Fig. 1. A stock solution of 27 mM veratridine (Aldrich) in ethanol was diluted 1:10 with incubation medium and 10 μ l of the latter solution were injected into the cuvette, giving a final concentration of 5×10^{-5} M veratridine. TTX in incubation medium was injected to give a final concentration of 4×10^{-7} M. Control injections of equivalent concentrations of ethanol did not promote chemiluminescence and a preliminary study showed that neither veratridine nor tetrodotoxin interfered with the response of the luciferin-luciferase system to added ATP.

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Role of electrogenic sodium pump in slow synaptic inhibition is re-evaluated

THE mechanism of synaptic transmission at most synapses involves an increase in conductance of the postsynaptic membrane to certain ions^{1,2}. Certain slow postsynaptic inhibitions in both the central and peripheral nervous systems are, however, not generated in this way³⁻¹³. Nishi and Koketsu³⁻⁵ proposed that the slow inhibitory postsynaptic potential (IPSP) in bullfrog sympathetic ganglia is due to a unique mechanism, the synaptic activation of the electrogenic Na pump (see also refs 11-15). In many, if not all, neurones, the outward pumping of sodium is coupled to the inward transport of K, with more Na extruded than K taken in, thus producing an outward movement of positive charge¹⁶. This electrogenic pumping of Na, which is blocked by ouabain or K-free Ringer, produces a hyperpolarisation of the membrane without a change in membrane permeability¹⁶. In the present experiments, the direct role of the electrogenic Na pump in slow synaptic inhibition was tested. The experiments were carried out on the ninth or tenth paravertebral sympathetic ganglion of the bullfrog using the sucrose gap technique. We found that although electrogenic Na pumping was inhibited by

ouabain or K-free Ringer, the slow IPSP was not blocked by ouabain and was enhanced by K-free Ringer. The data are consistent with the hypothesis that the slow IPSP may result from an inactivation of Na conductance rather than from an activation of the electrogenic Na pump.

In mammalian sympathetic ganglia, the administration of nicotinic cholinergic agonists produces a brief depolarisation due to an increased conductance to Na^+ and K^+ . This is followed by an after-hyperpolarisation that results from the electrogenic extrusion of accumulated intracellular Na^+ ions¹⁷⁻¹⁹. We used this nicotinic after-hyperpolarisation to characterise the inhibition of electrogenic Na pumping in frog sympathetic ganglia by ouabain and K-free Ringer. Figure 1*a* (middle) shows that K-free Ringer rapidly inhibits the after-hyperpolarisation. This inhibition persisted as long as the preparation was bathed in K-free Ringer, but was rapidly reversible (Fig. 1*a*, bottom). Inhibition of the after-hyperpolarisation by ouabain occurred more slowly. Figure 1*b* (middle) shows that the after-hyperpolarisation following an acetylcholine (ACh) depolarisation was abolished after 1 h in ouabain 10^{-6} M. This effect was only slowly reversible, with the restoration of the ACh after-hyperpolarisation

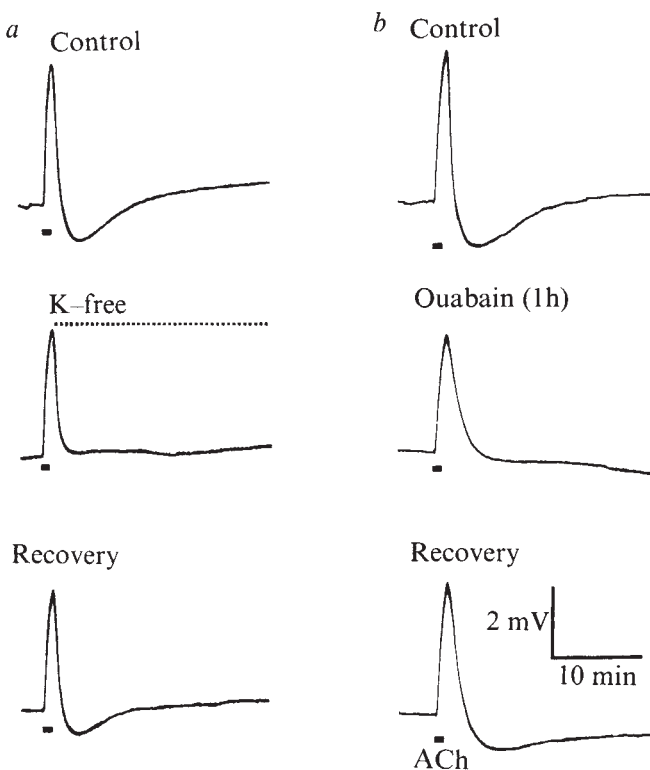


Fig. 1 Effects of K-free Ringer and ouabain on electrogenic sodium pumping. *a*, Effect of K-free Ringer: *top*, control response of sympathetic ganglion to administration of ACh (3 mM) for 1 min (bar) (note rapid depolarisation due to increased Na and K conductance followed by more prolonged after-hyperpolarisation resulting from electrogenic pumping of Na); *middle*, response when superfusion with K-free Ringer (dotted line) started at peak of ACh depolarisation (note block of after-hyperpolarisation); *bottom*, recovery of the after-hyperpolarisation following an ACh depolarisation (recorded 25 min after return to normal Ringer solution). *b*, Effect of ouabain: *top*, control response to administration of ACh (2.5 mM) for 1 min (bar) (as in *a*, but from a different preparation); *middle*, response to ACh after superfusion for 1 h with Ringer solution containing ouabain (10^{-6} M) (note block of the after-hyperpolarisation); *bottom*, recovery of the after-hyperpolarisation following an ACh depolarisation, after washing with normal Ringer's solution for 4 h 45 min. All records from a rectilinear pen recorder (Brush Model 280). Experiments were carried out on the ninth or tenth paravertebral sympathetic ganglion of the bullfrog using sucrose gap recording⁴. The preparation was continuously perfused with oxygenated Ringer solution of the following composition (mM): NaCl 100, KCl 2, CaCl_2 1.8, Tris HCl 16, pH 7.2, and glucose 1 g l⁻¹. K-free Ringer had the same composition except that KCl was omitted.

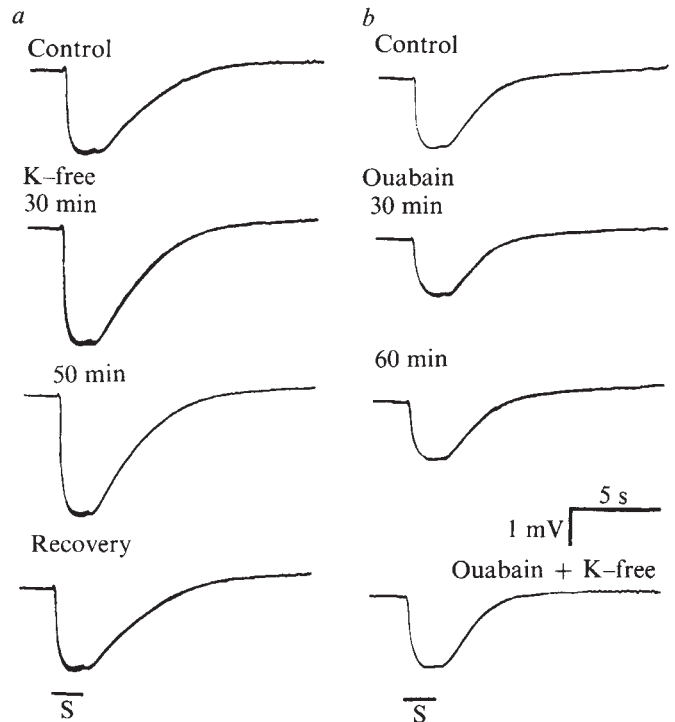


Fig. 2 Effects of K-free Ringer and ouabain on the slow IPSP. *a*, Effect of K-free Ringer: *top*, control record of slow IPSP elicited by repetitive stimulation of the eighth spinal nerve with 1-ms pulses at a frequency of 50 Hz (2-s period of stimulation is indicated by the line labelled S under bottom record; nicotine 30 μM used throughout the experiment to antagonise the fast EPSP); *middle*, slow IPSPs recorded 30 and 50 min after beginning superfusion of ganglion with K-free Ringer (note increase in slow IPSP amplitude in K-free Ringer); *bottom*, recovery of slow IPSP recorded 25 min after return to normal Ringer; *b*, Effect of ouabain: *top*, control record of slow IPSP (as in *a* except from a different preparation; nicotine (30 μM) was used throughout the experiment to antagonise the fast EPSP); *middle*, slow IPSPs recorded 30 and 60 min after beginning superfusion with Ringer solution containing ouabain (10^{-6} M) (note small reduction in slow IPSP amplitude compared with control); *bottom*, slow IPSP after 20 min superfusion with K-free Ringer containing ouabain (10^{-6} M) (record taken after 90 min in ouabain (10^{-6} M), with 30 and 60 min records shown above; note increase in slow IPSP amplitude compared with 30- and 60-min records). Records from photographs of oscilloscope traces.

taking 4 h or more (Fig. 1*b*, bottom). These results indicate that K-free Ringer and ouabain are effective inhibitors of the electrogenic pumping of Na in the bullfrog sympathetic ganglion. We therefore used these agents to test the hypothesis that the slow IPSP is generated by the activation of the electrogenic Na pump.

Figure 2 illustrates the effects of K-free Ringer and ouabain on the slow IPSP. The application of K-free Ringer solution produced little or no change in resting membrane potential, but resulted in a rapid and persistent increase in the amplitude of the slow IPSP. Figure 2*a* illustrates the increase in slow IPSP amplitude after 30 and 50 min in K-free Ringer. To investigate the muscarinic slow IPSP, the nicotinic fast excitatory postsynaptic potential (EPSP) must be antagonised. When nicotine (30 μM) was used to antagonise the fast EPSP, 1 h in ouabain (10^{-6} M) resulted in a partial (10–40%) reduction in slow IPSP amplitude (Fig. 2*b*). When d-tubocurarine (70 μM) was used as the nicotinic antagonist, however, no significant reduction was observed after 1 h in ouabain (10^{-6} M). Figure 2*b* (bottom) shows that even after 90 min in ouabain, the administration of K-free Ringer still increased the amplitude of the slow IPSP.

Two hypotheses have been advanced to explain the electrogenesis of the slow IPSP. One proposes that the

slow IPSP is due to the activation of the electrogenic Na pump³⁻⁵. The present experiments show that although K-free Ringer and ouabain inhibit the electrogenic pumping of Na, they do not block the slow IPSP. The other hypothesis proposes that the slow IPSP is generated by a decrease in resting Na conductance⁷. This mechanism would readily explain the effects of ouabain and K-free Ringer in the present experiments. When the fast EPSP was antagonised by nicotine, the slow IPSP was partially reduced in amplitude after 1 h in ouabain. It is known that nicotine can increase resting Na conductance³⁰, and in the presence of ouabain, which inhibits the extrusion of Na⁺, an accumulation of intracellular Na would be expected¹⁹. This accumulation would decrease the Na gradient and thus reduce the amplitude of a slow IPSP generated by a decrease in resting sodium conductance^{3,7}. This interpretation is supported by the observation that when *d*-tubocurarine, a nicotinic antagonist that does not increase resting Na conductance³¹, was used, ouabain did not produce a significant reduction in slow IPSP amplitude. The enhancement of slow IPSP amplitude by K-free Ringer is difficult, if not impossible, to explain in terms of the electrogenic Na pump hypothesis, since the presence of extracellular K⁺ is necessary for the coupled extrusion of Na⁺ (ref. 16). On the other hand, such an enhancement is predicted by the inactivation of Na conductance hypothesis because the driving force for the slow IPSP is the K equilibrium potential, E_K (ref. 7), which would be increased in K-free Ringer, thus increasing the amplitude of the slow IPSP. Furthermore, if the slow IPSP were due to electrogenic Na pumping, the combination of ouabain and K-free Ringer would be expected to result in a greater reduction in slow IPSP amplitude. The observed enhancement (Fig. 2b), however, can be explained by the Na inactivation hypothesis, as discussed above. The present experiments suggest that the slow IPSP is not due to the synaptic activation of the electrogenic Na pump. On the other hand, the results can be readily explained by and support the hypothesis that the slow IPSP is generated by an inactivation of resting Na conductance.

The electrophysiological properties of the slow IPSP (ref. 7) are remarkably similar to both the hyperpolarising response of vertebrate photoreceptors to light²²⁻²⁴ and central neurones to noradrenaline⁸⁻¹⁰. The conductance decrease mechanism may therefore be of general significance in understanding the generation of slow inhibitions in the nervous system. Furthermore, this mechanism may have important functional consequences. In central neurones, such a hyperpolarisation of the membrane would inhibit neuronal discharge; the concomitant increase in membrane resistance, however, might be expected to increase the amplitude of convergent EPSPs (refs 25 and 26). This could result in an improved signal to noise ratio for convergent synaptic excitation.

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PETER A. SMITH
FORREST F. WEIGHT

Laboratory of Neuropharmacology,
National Institute of Mental Health,
Saint Elizabeths Hospital,
Washington, DC 20032

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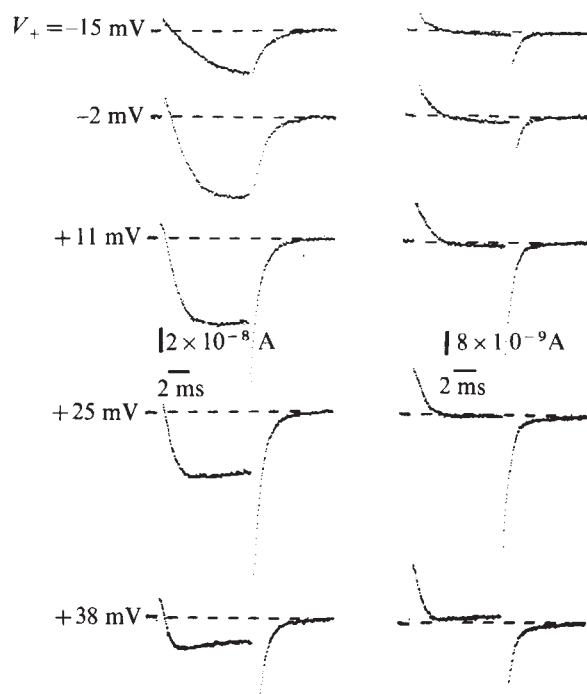
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Asymmetrical displacement currents in nerve cell membrane and effect of internal fluoride

We have recorded asymmetrical displacement currents which may be linked to the activation of calcium permeability in the membrane of isolated snail neurones. The search for these currents was prompted by two lines of evidence. First, the existence of 'gating current' associated with sodium channel activity¹. And second, the existence of voltage-dependent charge movement in muscle fibres, which has some role in excitation-contraction coupling² and is supposedly involved in the process of calcium release³. An attempt to measure both Ca and Na gating currents in *Aplysia* neurones has been reported⁴.

We measured asymmetrical transients in neurones isolated from the snail *Helix pomatia*. The neurones were internally dialysed with potassium-free 130 mM Tris-phosphate solution + 5 mM CsCl (pH 7.4). The external solution contained 120 mM Tris-HCl and 10 mM Ca and was free of Na, K and Mg. External cadmium ions were extremely effective in blocking

Fig. 1 Traces of calcium inward current (10_+ , 10_- ; left column) and corresponding asymmetrical transients (50_+ , 50_- ; right column). For the right column the Ca inward current was blocked by 2 mM Cd. The test potentials are indicated. Current calibrations were calculated for a single sweep. Holding potential -60 mV.



calcium inward current (K_d 7.2×10^{-5} M) (ref. 5). Therefore 2 mM CdCl_2 was added to the external solution to ensure complete and reversible block of the calcium current. All experiments were performed at 20–22 °C.

We used a modified version of the intracellular dialysis method described before⁶, including voltage clamp improvement, in this investigation. The main purpose of the modification was to increase the rate of the capacitive charging process. The isolated cell being fixed in the pore was impaled with a potential measuring microelectrode. This procedure minimised the error due to the series resistance. Further increase in the rate of response was achieved by a feedback technique suggested by Katz and Schwartz⁷, which gave better results than the conventional method of compensation for series resistance. Some experiments were performed without the additional feedback. The potential reached 95% of its final value in 150 μs with Katz and Schwartz compensation, in 350 μs with conventional compensation and in 1 ms with no additional feedback.

The calcium inward currents and asymmetrical transients were obtained in their net form after algebraic summation of membrane currents produced by an equal number of equal positive and negative voltage clamp pulses. Dwell time of the averaging was 50 μs . The number of pulses was usually 10 positive and 10 negative (10_+ , 10_-) for calcium currents and 50_+ , 50_- for asymmetrical transients. The interval between pulses was 1 s. In most cases positive and negative pulses were automatically alternated to ensure removal of slow inactivation. All kinds of symmetry tests were performed including averaging the pulses first from resting (-50 or -60 mV) and then from highly negative holding potential (between -100 and -150 mV). In the latter case negligible asymmetrical transients were obtained. The source of these transients has not yet been identified. They may be accounted for as the gating of fast potassium outward current, which is known to be inactivated with test pulses reaching -50 mV. The amount of charge transferred by the asymmetrical displacement current was calculated automatically by the averager which measured the area under the off-set line and then subtracted it from the area under the asymmetrical transient (for the 'on'-process) or subtracted the area under the asymmetrical transient from that under the base line (for the 'off'-process).

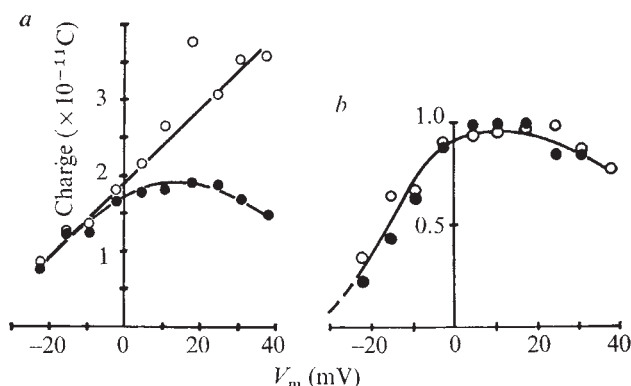


Fig. 2 *a*, Voltage dependence of charges Q_{on} (●) and Q_{off} (○) transferred by the displacement currents, calculated for a single sweep. *b*, Voltage dependence of Q_{on} (●) and calcium conductance g_{Ca} (○). Conductance was calculated for $V_{Ca} = 170$ mV (normalised). (Both *a* and *b* are from the same experiment as shown in Fig. 1).

Figure 1 shows a typical family of calcium inward current traces and corresponding asymmetrical transients obtained after cadmium block. The small inward current noticeable in the asymmetrical transient trace is due to rectification of the leakage current. The increase in test potentials above +20 mV led to the appearance of a time-dependent nonspecific outward current component. The presence of this component probably contaminated the asymmetrical transients at high test potentials by increasing the charge transferred during the 'off' pulse (Q_{off}). This is shown in Fig. 2*a* where the voltage dependence of Q_{on} and Q_{off} is presented. Q_{on} reached a peak and then tended to diminish, whereas Q_{off} persistently increased. Nevertheless, the equality of Q_{on} and Q_{off} at low test potentials allows us to identify the asymmetrical transients as displacement currents.

Figure 2*b* shows the voltage dependence of both g_{Ca} and Q_{on} . g_{Ca} was calculated as $I_{Ca}/(V - V_{Ca})$. To avoid the effect of the nonspecific outward current we obtained the reversal potential V_{Ca} from the instantaneous $I-V$ relationship at moderate test potentials. It varied between 130 and 200 mV

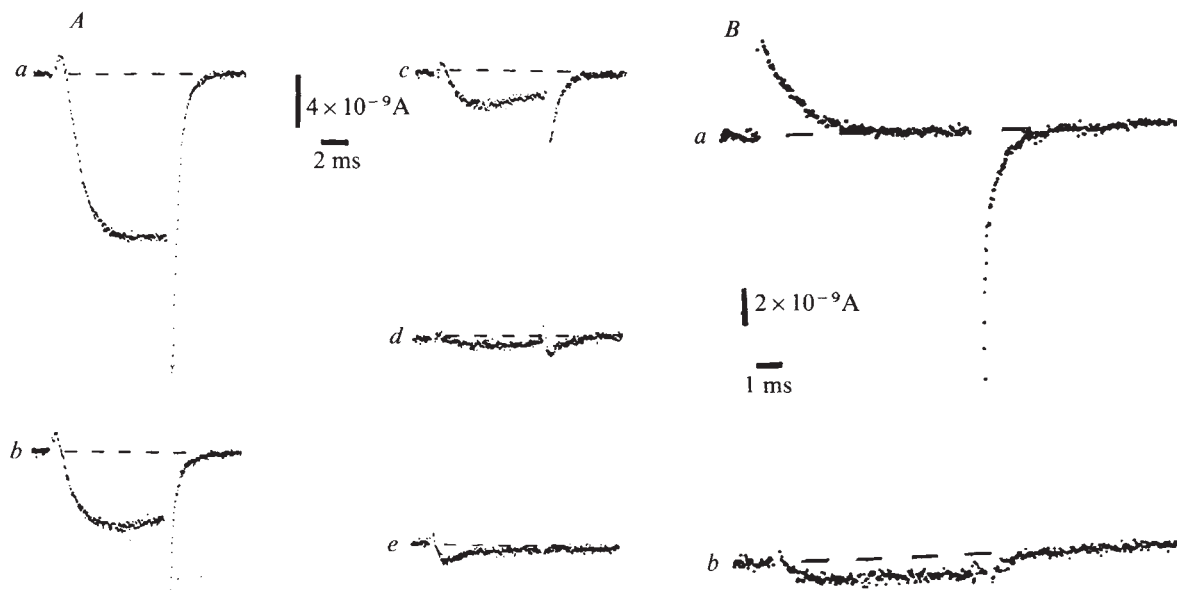


Fig. 3 *A*, The effect of fluoride on calcium inward current (*a-d*; $V_t + 8$ mV; 10_+ , 10_-) and on the displacement current, with conventional compensation for series resistance. Averaging was performed first with Tris-phosphate inside (*a*), and then every 2 min after the application of fluoride (*b-d*). Sodium inward current ($V_t - 19$ mV; 10_+ , 10_-) was observed after the return to normal Ringer (*e*). Holding potential -50 mV. *B*, The effect of fluoride on the displacement current. Record before (*a*) and after (*b*) adding fluoride. The feedback increasing the rate of response was introduced. Holding potential -50 mV. The negative off-set line in *b* is due to the decrease of outward going leakage current caused by internal fluoride. $V_t + 6$ mV; 50_+ , 50_- .

for different cells. The value of V_{Ca} thus measured corresponds to that obtained for internally perfused barnacle muscle fibres⁸. g_{Ca} and Q_{on} have a similar potential dependence (Fig. 2b). This similarity, in fact, is not critically dependent on the V_{Ca} value. A decrease of Q_{on} and g_{Ca} at strong positive potential, although observed in the same potential region, could be only apparent: (1) the I_{Ca} values could be underestimated because of the nonspecific time-dependent current activation, and (2) a certain part of Q_{on} could be lost in the initial blank period because of the faster kinetics of the 'on'-process at more positive membrane potentials.

Like the calcium inward currents, the displacement currents were inactivated at more positive holding potentials. This inactivation was very pronounced, beginning with holding potential of -30 mV.

The substitution of internal phosphate by fluoride anions caused a complete and irreversible depression of asymmetrical displacement currents. It was accompanied by a decrease of outwardly directed leakage. Figure 3A demonstrates simultaneous reduction of the calcium inward current and of the initial displacement current. In this experiment the conventional compensation for series resistance was used. The delayed establishment of the final potential level led to the appearance of a rising phase in the time course of displacement current development. It is probable that the very slow rising phase of the calcium gating current described for *Aplysia* neurones⁴ is caused in a similar manner.

A small sodium inward current which was not blocked by fluoride could be detected in this cell after the return to normal Ringer (Fig. 3A). Its time course demonstrates that asymmetrical transients associated with sodium channel activation are completely lost in the 250- μ s blank period. Figure 3B shows a similar experiment but for displacement currents only and with the additional feedback.

Our data suggest that the selective block of calcium currents by internal fluoride is caused by irreversible damage to the gating mechanism of calcium channels.

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P. G. KOSTYUK
O. A. KRISHTAL
V. I. PIDOPLIHKO

*Bogomoletz Institute of Physiology,
Ukrainian Academy of Sciences,
Kiev, 252601, GSP, USSR*

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Does cyclic GMP mediate the negative inotropic effect of acetylcholine in the heart?

DURING vagal stimulation the pacemaker activity of the heart is diminished. The reduction in heart rate is due to a release of acetylcholine (ACh) from the parasympathetic nerve terminals that increases the permeability of the myocardial cell membrane for potassium ions (for review see ref. 1). This is accompanied by a shortening of the action potential duration in atrial muscle and a diminished calcium uptake², which in turn results in a negative inotropic effect. Voltage clamp experiments in mammalian atrial muscle have shown that with higher concentrations of ACh not only is the potassium current augmented but also the slow inward current of calcium is reduced³. It is

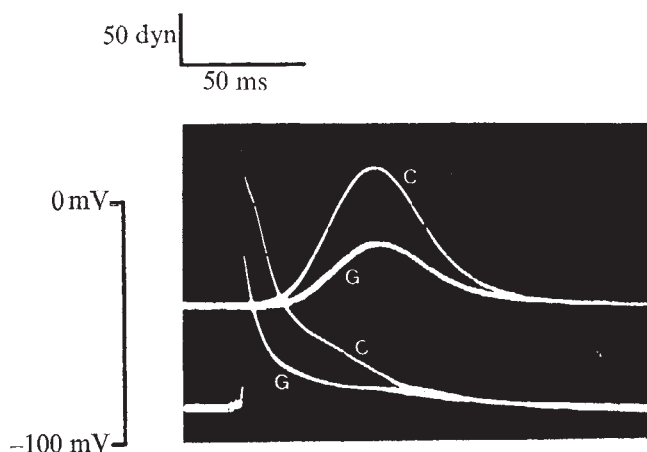
not clear how the current changes brought about by ACh are mediated. I have described the negative inotropic action of 8-bromo-cyclic GMP in the mammalian heart⁴, and these findings were confirmed in ventricular fibre fragments from mouse hearts⁵. If cyclic GMP levels in the myocardial cell are linked to the effects of ACh, as George *et al.* proposed⁶, then 8-bromo-cyclic GMP in addition to its negative inotropy should produce changes in the ion movements similar to those described for ACh. I report here the effects of 8-bromo-cyclic GMP on the action potential and the presumed underlying ion movements in rat atrial muscle.

Figure 1 shows the effects of 8-bromo-cyclic GMP on isometrically developed tension and on action potential configuration. Force of contraction was reduced to about 40% of control after 60 min; at the same time, the action potential duration was shortened at all voltage levels. Whereas the upstroke of the action potential is thought to be caused by an inflow of sodium ions⁷, the repolarisation phase is determined mainly by an outflow of potassium ions⁸. During the time course of repolarisation the potential is held at more positive potentials by an inwardly directed flow of calcium ions that has been reported for different species in ventricular and atrial preparations (for review see ref. 9). This slow inward current of calcium is believed to be of major importance for the initiation and maintenance of contraction^{10,11}.

The most direct way of testing any ion movements across cellular membranes is the use of radionuclides. Figure 2 shows that the calcium uptake in beating preparations was reduced in the presence of 8-bromo-cyclic GMP. The extent of the calcium inflow during the cardiac action potential could be diminished directly by a change in the conductance for this ion. Indirectly it could be diminished by a change in the currents of sodium and potassium that could either delay or prevent full activation of the calcium conductance by a decreased sodium inflow, or turn off and accelerate the inactivation of the calcium conductance by an increase in potassium outflow. The latter is thought to be true for low concentrations of ACh³. Either of these current changes would be detected by following the ion movements of sodium and potassium.

The uptake of radioactive sodium (²²Na, determined by liquid scintillation counting) or the net content of sodium (electrolyte contents were determined by atomic absorption spectrophotometry) were not altered in the presence of 8-bromo-cyclic GMP. Therefore the negative inotropic

Fig. 1 Effects of 10^{-4} M 8-bromo-cyclic GMP on isometric force (middle traces) and transmembrane action potential (other traces) in a rat left auricle after 60 min. The preparation was driven electrically at 0.2 Hz; other conditions were as described earlier⁴. The membrane potential was recorded intracellularly by the use of glass microelectrodes filled with 3 M KCl. C, control; G, 8-bromo-cyclic GMP.



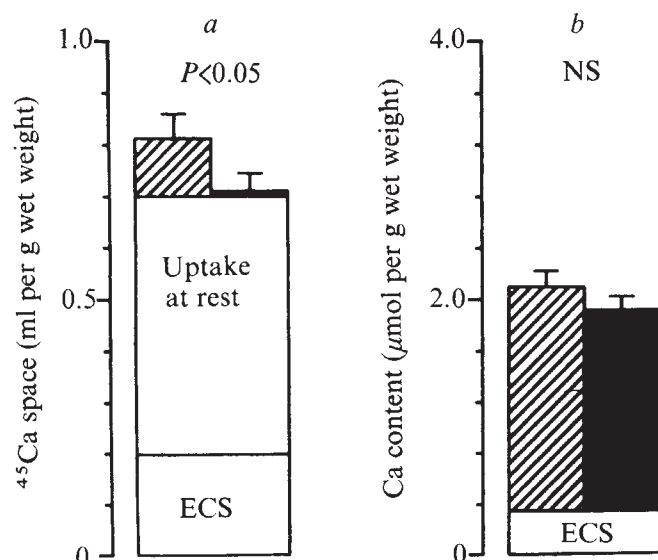


Fig. 2 Effects of 10^{-4} M 8-bromo-cyclic GMP on ^{45}Ca uptake (a) and Ca net content (b) in resting or beating (2 Hz) rat left auricles. Hatched columns, control; filled columns 8-bromo-cyclic GMP. Radioactivity was determined by liquid scintillation spectrometry after the preparations had been dissolved in 0.5 ml of tissue solubiliser, and 1 ml of 1 N HCl, plus 10 ml of scintillation fluid, had been added. The ^{45}Ca space was calculated according to Grossman and Furchgott². Each column represents the means $\pm$ s.e. of eight preparations exposed for 15 min to ^{45}Ca after 30 min of stabilisation with or without 8-bromo-cyclic GMP. All values were obtained in individual preparations. The difference in ^{45}Ca uptake was not due to a change in the extracellular space (ECS, determined by the ^{14}C -inulin method, $n = 8$); also, the uptake of ^{45}Ca while at rest was not different in controls and preparations treated with 8-bromo-cyclic GMP, $n = 16$. The measurement of the transmembrane influx of calcium related to contraction is hindered by the influence of the extracellular space and the large amount of calcium taken up while at rest. These difficulties have been described by Grossman and Furchgott². The representation of resting uptake and extracellular space should demonstrate the large influence of these 'background values' on the measurements in beating preparations. Standard errors were omitted for clarity because these values were obtained in different preparations and not considered in the statistical evaluation of results. The net content of calcium was determined by atomic absorption spectrophotometry in 16 preparations, following the same time schedule and then ashing the preparations in a 1:1 mixture of HNO_3 : HClO_4 at 180°C and redissolving the ash in N/10 HCl solution containing 1% LaCl_3 .

action of 8-bromo-cyclic GMP does not seem to be accompanied by any change in sodium movements. Indeed, a change in ion fluxes was more expected with respect to potassium, because ACh is known to induce changes in the movements of this ion. Figure 3 shows, however, that potassium efflux did not change in the presence of 8-bromo-cyclic GMP; also, the net content of potassium was not significantly affected. The efflux curve of radioactive potassium in control muscles was virtually identical to that obtained in the presence of 8-bromo-cyclic GMP and was best fitted by the equation

$$\ln Y = \ln(100 - 0.031t)$$

In resting preparations, there was also no difference in the rate of efflux of ^{42}K with or without 8-bromo-cyclic GMP. The rate constant of potassium extrusion was slightly smaller than in beating preparations and amounted to $0.026 \pm 0.001 \text{ min}^{-1}$ (mean $\pm$ s.e., $n = 8$). In identical conditions, as described in the legend to Fig. 3, ACh increased the rate of potassium extrusion by a factor of about 1.3–1.4 (Fig. 4). Because maximally effective concentrations of both 8-bromo-cyclic GMP and ACh were used in the ^{42}K washout experiments, a quantitative difference in the cyclic nucleotide and acetylcholine sensitivity is unlikely.

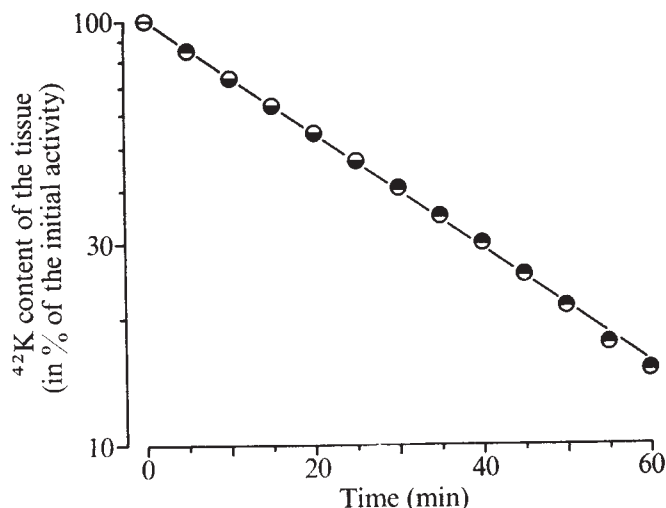
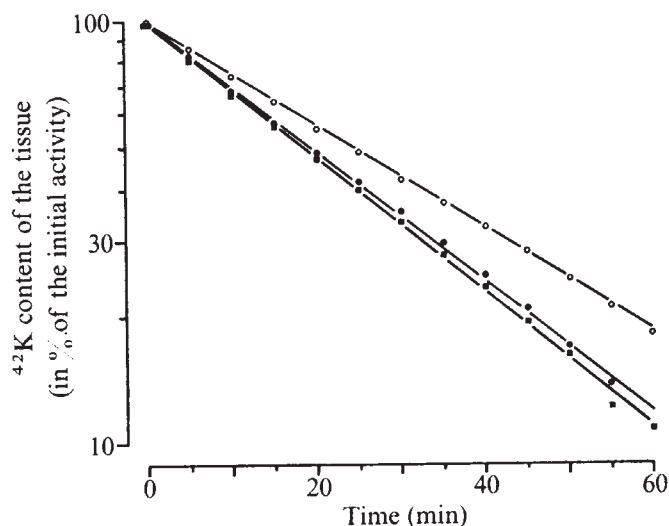


Fig. 3 Effects of 10^{-4} M 8-bromo-cyclic GMP on ^{42}K efflux in rat left auricles electrically stimulated at 2 Hz. $\circ$, Control; $\bullet$, 8-bromo-cyclic GMP. Radioactivity was determined by liquid scintillation spectrometry and by γ -scintillation counting. Symbols represent means $\pm$ s.e. of six preparations each (the values including s.e. did not vary to a greater extent than the size of the symbols used). Control and test values were obtained in individual preparations that were loaded with ^{42}K for 90 min first; then, the amount of ^{42}K released by the muscle into non-radioactive Tyrode solution was determined each 5 min; after 60 min, the experiment was finished and the residual activity in the muscle was determined. The ^{42}K content of the muscle was calculated by cumulative addition of all released amounts of radioactivity for any time the ^{42}K effluent from the preparation had been determined. The theoretical curve (the equation is given in the text) was obtained from plots of log concentration against time, using the method of least squares.

The results show that the negative inotropic effect of 8-bromo-cyclic GMP can be explained solely on the basis of an alteration in the calcium influx in beating preparations. A corresponding change is seen in the configuration of the action potential. There is no indication that the potassium system is affected by 8-bromo-cyclic GMP in the way identified for ACh. Given that in voltage clamp experiments ACh also reduced the slow inward current of

Fig. 4 Effects of ACh on ^{42}K efflux in beating rat left auricles. $\circ$, Control; $\bullet$, 10^{-6} M ACh; $\blacksquare$, 5×10^{-6} M ACh. Experimental procedure was as described in Fig. 3. Means $\pm$ s.e. of rate constants of the washout process amounted to 0.028 ± 0.002 (control, $n = 4$), 0.036 ± 0.003 (10^{-6} M ACh, $n = 3$) and 0.038 ± 0.003 (5×10^{-6} M ACh, $n = 4$) min^{-1} . Correlation coefficients for all experiments varied between 0.996 and 0.999. Significance level for the difference between the washout curves obtained in control conditions and ACh was $P < 0.05$.



calcium in mammalian³ and frog^{12,13} atrial muscle, it is possible that the effects of ACh are mediated in part by changes in the intracellular levels of cyclic GMP; on the other hand, another pathway by which ACh mediates its physiological effects seems to be obvious.

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HERMANN NAWRATH

Department of Pharmacology,
University of Mainz,
6500 Mainz, FRG

Received 26 August 1976; accepted 15 March 1977.

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Variation of thin filament length in heart muscle

ALTHOUGH the length dependence of force generation by heart muscle has been attributed to the degree of overlap of thin and thick filaments within the sarcomere, the required accurate values of the lengths of cardiac thin filaments have not been available. The existence in frog atrial trabeculae of thick filaments of uniform 1.55 μm length, of a small number of very long thin filaments, and of rosettes containing fewer or more than six thin filaments surrounding each thick filament has been noted³ (Fig. 1), but no study of the dimensions of a statistically significant number of thin filaments has been made. Using serial transverse electron micrographs of cardiac cells we have found that the lengths of thin filaments vary by as much as 0.6 μm within a single sarcomere in a single myofibril. All other factors being constant, this broad range should increase the length of the plateau of the sarcomere length–tension relationship and allow the myocardial cells contracting *in situ* to operate entirely on the plateau. The sliding of

Fig. 1 Electron micrograph of a transverse section of rat atrial muscle. A small number of long thin filaments extends into the hexagonal M bridge and thick filament array. Thick filaments are surrounded by 0–6 thin filaments. Total magnification: $\times 121,000$.

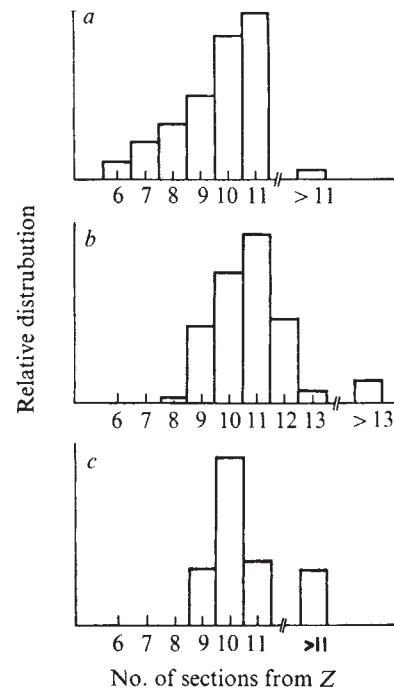
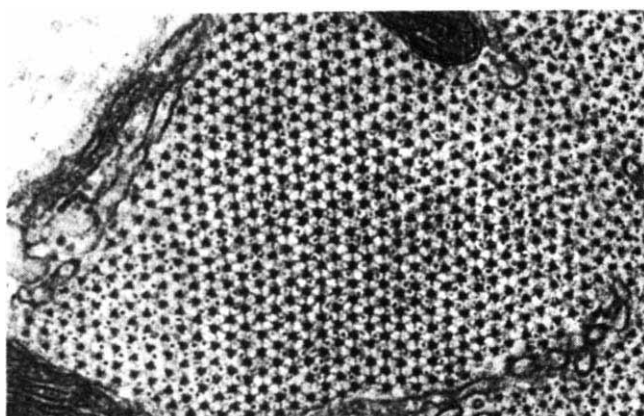


Fig. 2 Relative frequency of thin filament lengths which are expressed in number of sections from the flat Z band for (a) rat atrial trabeculae, (b) frog atrial trabeculae, and (c) rat papillary muscle. The estimate of the section thickness was 0.1 μm whether it was based on the pale gold interference colour of the freshly cut, floating sections or derived from the known 1.5 μm length of the thick filament, which was used as an internal calibration standard that compensated for any generalised filament shrinkage⁹. The width of each histogram bar represents the section thickness and thus the limit of resolution of the technique.

myofilaments, therefore, should have little direct effect on force generation in the intact heart.

We have measured the lengths of populations of thin filaments in frog and rat atrial trabeculae and rat papillary muscle by analysing electron micrographs of serial transverse sections through a major portion of sarcomeres of muscles which were stretched and held throughout the preparative procedures of fixation and dehydration. Two different regions from each tissue were studied. Longitudinal sections are unsuitable for estimating filament length because in thick sections there is no discernible H zone, and in very thin sections there are the dangers of producing errors by either clipping off part of the filaments or superposing filaments. To eliminate artifacts associated with the use of the approximately 0.1 μm thick transverse serial sections, regions were used only when the Z band was flat and entirely included within one section; when all filaments were absolutely perpendicular to the plane of section without using any tilting of the specimen stage; and when at least 400 thin and 200 thick filaments could be followed in a given region from one section to another. The numbers of thin and thick filaments were then counted in each transverse section and the relative lengths of thin filaments from the Z band deduced from the ratios of thin to thick filaments. Estimates of absolute values for filament lengths were determined by multiplying the number of serial sections through which the filaments extended by 0.1 μm , the approximate thickness of a section. Similar values were produced by using the length of the thick filament as an internal calibration. Values for the ratios of the number of thin to the number of thick filaments varied between 0 and 2. The maximum value of 2 attests to the suitability of the sarcomere for filament length analysis; a value greater than 2 would indicate poor lateral alignment of thick filaments, whereas a value less than 2 would show that some of the shortest thin filaments did not reach the A band

lattice as a result of overstretching of the muscle. Since the absolute number of thick filaments is constant throughout the A band, the relative numbers of thin filaments of each length have been obtained from a comparison of the ratios of thin to thick filament densities in each section. Typical data are shown in Fig. 2.

Thin filament lengths in frog atrial trabeculae varied from eight to more than thirteen section thicknesses, a span of more than $0.6\ \mu\text{m}$, and in rat atrial trabeculae the range was equal to over six section thicknesses, or more than $0.6\ \mu\text{m}$. In the rat papillary muscle, however, thin filaments differed in length from nine to more than eleven section thicknesses, or about half the spread of atrial tissue. The wide range of thin filament lengths found within a single sarcomere of a single myofibril indicates that there is no unique value for the extent of overlap even within one sarcomere. Consequently, any relation between length and force generation will be dependent not only on sarcomere length but also on the distribution of lengths of thin filaments.

An assessment of the effects of the broad distribution of lengths of thin filaments on the relationship between sarcomere length and developed force can be made if the data are coupled with the observations of Gordon *et al.*⁵, relating force to certain relative positions of thick and thin filaments. The calculations involved construction of sarcomere length-tension curves for each thin filament length, weighting by the appropriate factor from the length distribution, and summation of all the weighted curves to give a composite, calculated sarcomere length-developed tension curve. The exact basis for the corner between the ascending limb and the plateau has not been well defined;

the decrease in developed tension with sarcomere shortening past the plateau might be due to such things as interference of cross-bridge activity in the vicinity of a larger than usual number of actin filaments or the appearance of an internal resistance from the mutual interaction of thin filaments from opposite sides of the sarcomere during double overlap. Separate calculations based on decreased generation of force from either the sliding of a thin filament past the bare zone and into the opposite half of the A band or the double overlap of thin filaments from opposite sides of the sarcomere produced only slightly different results.

Figure 3a and b show the relations that were calculated using the first and simpler of the two modelling possibilities. The composite sarcomere length-developed tension curves of the atria have shapes distinctly different from that of skeletal muscle, particularly with respect to their much broader plateau regions. The wide range of thin filament lengths in atrial muscle provides a nearly constant degree of functionally effective thin and thick myofibril overlap over the physiological working range, and the sliding of filaments with changes in sarcomere length should therefore have little direct effect on the generation of tension.

The presence of a broad zone with only a very shallow slope in the length-tension relationship of calcium-activated, mechanically skinned cardiac fibres⁷ is consistent with the observed dimensions of thin filaments, and the absence of a plateau for the same relation in isolated cardiac muscles^{8,9} indicates that the modulation of force in the intact tissue must result from another step in the contractile cycle, such as activation⁷.

Of additional interest in this study was the correlation between the compliance of the tissue and the breadth of the distribution of thin filament lengths (Fig. 2); the stiffer papillary muscle had the narrower distribution. This raises the possibility that the breadth of the distribution may actually be set by the cell according to the amount of shortening which the cell undergoes in the intact heart to produce a plateau of adequate length in the sarcomere length-tension curve.

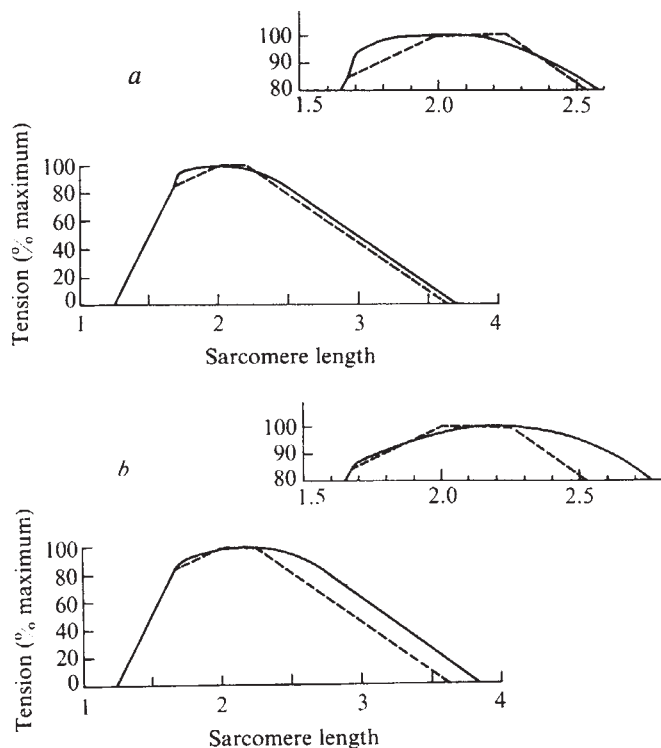
THOMAS F. ROBINSON
SAUL WINEGRAD

Department of Physiology,
School of Medicine,
University of Pennsylvania,
Philadelphia, Pennsylvania 19174

Received 20 December 1976; accepted 8 March 1977.

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Fig 3 Derived sarcomere length-developed tension curves (—) for (a) rat atrial trabeculae and (b) frog atrial trabeculae. Enlargements of the top portions of the curves appear in the corners. The derived relationship is the composite of individual sarcomere length-tension curves weighted by the appropriate factor from the distribution histogram (Fig. 2). Each of the individual curves was constructed for a given thin filament length using the inferences of Gordon *et al.*⁵, concerning the relationship between developed force and filament configuration. The plateau or near-plateau regions are about $0.7\ \mu\text{m}$ wide, compared with $0.20\text{--}0.25\ \mu\text{m}$ for skeletal muscle (-----).



Sodium sensitivity of baroreceptors mediates reflex changes of blood pressure and urine flow

CARDIOVASCULAR baroreceptors are sensitive to vascular pressures and their input to the central nervous system is essential for the regulation of arterial blood pressure. They also contribute to the regulation of extracellular fluid volume, because their reflex effects upon arterial pressure and renal blood flow have marked consequences for urine flow^{1,2}. Since they are mechanoreceptors their properties may be affected by sodium and potassium ions³⁻⁵. Lowering extracellular Na^+ (Na_o^+) increases the threshold pressure at which arterial baroreceptors begin to discharge and reduces their sensitivity to suprathreshold pressures^{6,7}. We therefore investigated the reflex effects upon arterial blood pressure

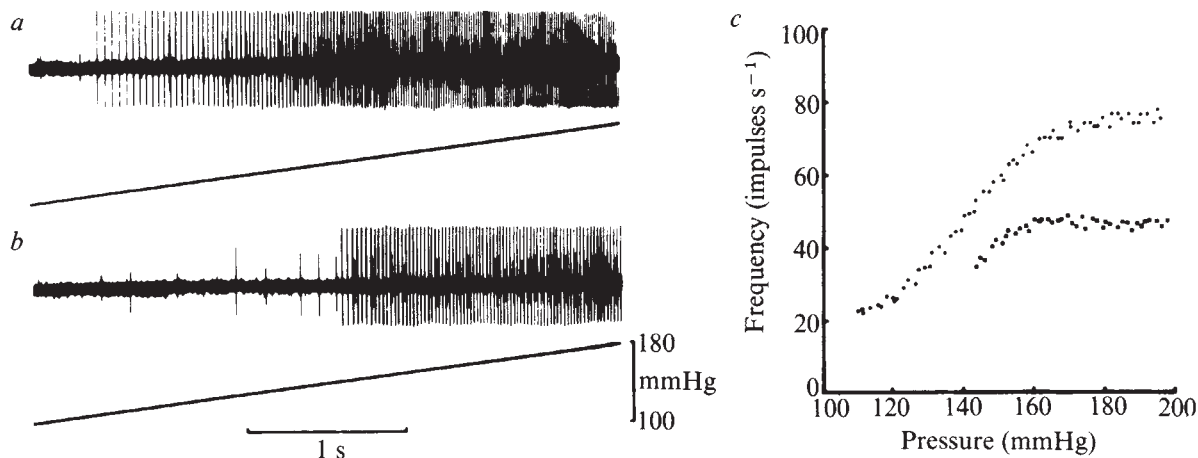


Fig. 1 Effects of lowering extracellular Na⁺ (Na_o⁺) on baroreceptor discharge. *a*, The tall spikes in the top trace are the neural discharge of a single baroreceptor in control solution and the slowly rising line in the bottom trace is a pressure ramp of 20 mmHg s⁻¹. *b*, Same arrangement as (*a*) but the baroreceptor is now in 50% Na_o⁺ solution. The threshold pressure is clearly increased and maximum discharge frequency is reduced. *c*, Discharge frequency-pressure plot of the neural discharge shown in *a* and *b*. Each point on the frequency against pressure plot is the inverse period between two successive spikes. Filled circles (left hand curve) and filled rectangles (right hand curve) represent the neural discharge patterns shown in (*a*) and (*b*), respectively.

and urine flow elicited by lowering the Na_o⁺ of carotid sinus baroreceptors. We report that reductions in Na_o⁺ of as little as 5% increase arterial blood pressure and urine flow. We propose that the sodium sensitivity of baroreceptors is important for their function as regulators of arterial blood pressure and body fluid volume.

Figure 1*a, b* shows the pressure-discharge curves of a single rat aortic arch baroreceptor in control and 50% Na_o⁺ solution. Composition of the control solution was, in mM: Na⁺, 145; K⁺, 6; Cl⁻, 127; Ca²⁺, 1.1; Mg²⁺, 1.2; HCO₃⁻, 25; H₂PO₄⁻, 1.2; SO₄²⁻, 1.2; dextrose, 5.5. Na_o⁺ was usually replaced by Tris [Tris (hydromethyl) aminomethane] at constant osmolality in the 95% and 87.5% Na_o⁺ solutions and at a measured increase of less than 1% in the 75% Na_o⁺ solutions and 2% or less in the 50% Na_o⁺ solutions. Neural discharge was elicited by applying pressure ramps of 4–20 mmHg s⁻¹ to the aortic arch. Slow ramp rates such as these generate pressure-discharge curves which are very similar to those elicited by steady state pressure steps⁷. Figure 1*c* shows the 'instantaneous' frequency versus pressure plots for the discharges and pressures shown in (*a*) and (*b*). Lowering Na_o⁺ clearly increases the pressure for threshold discharge

and reduces the sensitivity to suprathreshold pressures. Seventeen individual baroreceptors studied in this manner showed similar responses to reductions in Na_o⁺ of 25–50%.

The steady state pressure-volume relationship of the aortic segments used in these experiments was measured as described previously^{8,9}. The pressure-volume curves were unchanged by the test solutions so that the ionic actions were directly upon the receptors. In addition, the rate of rise of pressure in the isolated aortic segments elicited by fixed input ramp rates was unchanged in the test solutions.

The reflex experiments were performed on cats which had been in the vivarium for at least 2 d. They were fed Purina cat food and allowed to drink water freely. The animals were anaesthetised with a mixture of chloralose (Baker) 50 mg per kg body weight, and urethane (Matheson, Coleman & Bell), 200 mg per kg, injected intraperitoneally. The cervical vagi were cut bilaterally and one carotid sinus nerve was cut. The other carotid sinus was isolated and perfused via polyethylene tubing coupled to a Holter pump which was connected to a reservoir containing Krebs-Henseleit solution bubbled with 95% O₂ and 5% CO₂ and heated to 37 °C. The perfusate was collected from the external carotid artery so that none

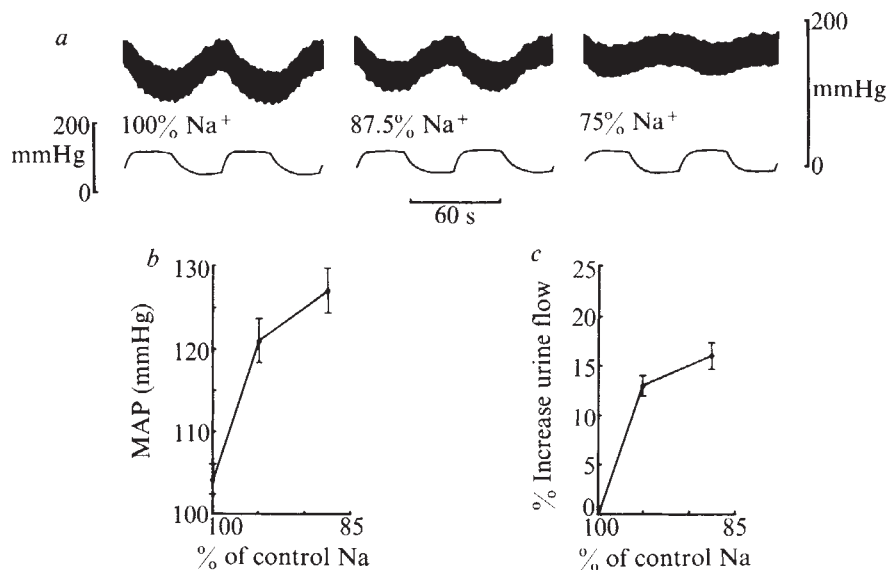


Fig. 2 Reflex effects of lowering Na_o⁺ in the carotid sinus perfusate on arterial blood pressure and urine flow. *a*, Top trace is systemic arterial blood (mean arterial) pressure (MAP) and bottom trace is left carotid sinus pressure. Repetitive step increases in sinus pressure elicit repetitive falls in arterial pressure. As Na_o⁺ in the carotid sinus is reduced at constant intrasinus pressure steps, mean arterial pressure rises and the oscillations in pressure elicited by constant steps are reduced. *b*, Mean arterial blood pressure as a function of carotid sinus Na_o⁺ at constant mean carotid sinus pressure. Data are from nine animals. *c*, Plot of the % increase in urine volume over control as Na_o⁺ is varied. The control urine flows were 39 ± 0.003 μl min⁻¹.

of it was added to the cat's circulating blood volume. Overdamped pressure steps could be superimposed by a parallel connection with a shaker pot (Ling 411) (Fig. 2a). Pressure in the perfusion circuit was measured using a Statham P23dB strain gauge manometer. Arterial blood pressure was measured by inserting a polyethylene catheter into a femoral artery. The catheter was connected to a Statham P23dB strain gauge manometer. The animal was given an intravenous injection of 0.9% NaCl equal in volume to 1.0% of body weight over 20 min at the beginning of the dissection. This was followed by continuous infusion of Krebs-Henseleit at 5 ml h⁻¹. Urine output was measured in timed fractions of 8–16 min duration by collecting the urine flowing from polyethylene catheters which were inserted into the ureters. Urine sodium concentration was measured using sodium glass miniature electrodes, or by an atomic absorption spectrophotometer (Instrumentation Laboratory IL253). The sodium glass was Corning NAS11-18, had a Nernstian response for Na⁺ and a selectivity of 50:1 and 100:1 respectively over K⁺ and NH₄⁺.

The isolated carotid sinus was perfused with the control solution until mean arterial blood pressure and urine flow became steady. This period was about 30 min. When the Na_o⁺ in the sinus perfusate was lowered to 95% or 87.5% at constant mean sinus pressure of 90–100 mmHg, mean arterial blood pressure always increased (Fig. 2b) and urine flow was always enhanced (Fig. 2c). The changes in arterial blood pressure elicited by applying overdamped pressure steps to the isolated carotid sinus were also reduced in amplitude (Fig. 2a). The relationship between the Na_o⁺ concentrations in the sinus perfusate and the changes from control of mean arterial blood pressure, and urine flow measured in nine animals, are shown in Fig. 2b and c. The increases in arterial pressure and urine flow evoked by the various test Na⁺ solutions were statistically significant at the 0.01 level. Recovery of blood pressure and urine flow to control values occurred between each test solution. The test solutions did not change the distensibility of the common carotid artery or the isolated carotid sinus and we conclude, as we did for rat aortic baroreceptors, that the ionic effects are due to a direct action upon the baroreceptors.

The role of the renal sympathetic nerves in the diuresis was examined in another six animals. One kidney in each animal was denervated and urine flow and sodium excretion were compared between the innervated and denervated kidneys of the same animal. Denervation did not affect control arterial blood pressure; a small but not significant increase in urine flow did occur¹⁰ (Fig. 3). Na⁺ excretion which varied considerably was reduced but the change was not significant (Fig. 3). Lowering Na_o⁺ to 87.5% in the isolated sinus always increased arterial blood pressure and urine flow and the increases were statistically significant. The diuresis was not different, however, between the innervated and denervated kidneys. Sodium excretion was not altered significantly by the test sinus solutions (Fig. 3).

The action of reduced Na_o⁺ upon the carotid sinus baroreceptors is not due to an osmotic effect since the osmotic pressure was unchanged by Tris substitution for Na_o⁺ at the 5% and 12½% levels and only slightly increased at 25% and 50% levels. Nor is the effect likely to be related to an action of Tris since replacement of 5% or 12½% NaCl with isosmotic sucrose (four experiments) gave the same response as substituting Tris for Na⁺. It is possible that lowering Na_o⁺ inhibited carotid body afferent discharge. The reflex effects on blood pressure would tend to offset the reflex effects of reducing baroreceptor input so that the sodium sensitivity of the baroreceptor reflexes might be even greater than the present experiments

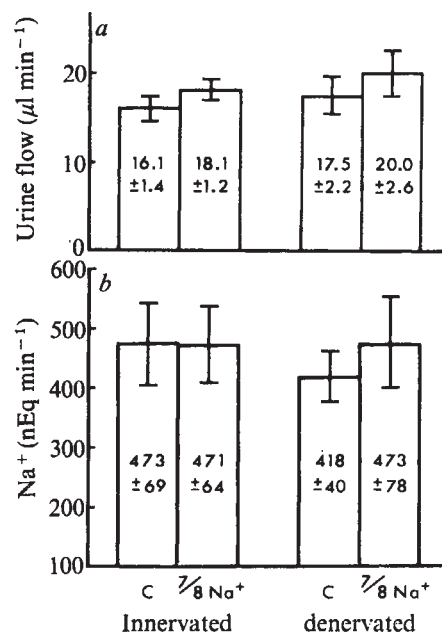


Fig. 3 Comparison between innervated and denervated kidneys of the effects of lowering carotid sinus Na_o⁺. a, The urine volumes are compared for innervated and denervated kidneys from six animals when the isolated carotid sinus was perfused with control solution, C, and when it was perfused with 7/8 Na_o⁺. There was a significant difference ($P < 0.01$) between the effects of control solution and 7/8 Na⁺ upon urine flow in both kidneys but there was no significant difference between innervated and denervated kidneys. b, The total urinary sodium output when the sinus is perfused with control and 7/8 Na⁺. Six animals were compared in this way. There were no significant differences between the effects of the two solutions on innervated or denervated kidneys.

indicate.

The sensitivity to Na_o⁺ was greater in the reflex experiments than in the individual baroreceptor discharge experiments⁷. This is undoubtedly due to the fact that in the reflex experiments all of the baroreceptors are tested simultaneously. The experiments using individual baroreceptors are complicated by the variability in threshold and sensitivity among different receptors as well as a 1–2% variability in individual receptors.

Arterial baroreceptors respond to small decreases in extracellular Na⁺ concentration by producing a reflex increase in arterial blood pressure and urine flow. Sodium excretion seems to be unchanged but, because of its variability, this point is not definitely established. The mechanism of the diuresis is unclear. The renal nerves do not seem to be essential and changes in antidiuretic hormone (ADH) secretion are unlikely to be involved since ADH is increased by reductions in baroreceptor discharge¹¹. The increase in arterial blood pressure is a likely possibility¹². Sodium sensitivity of baroreceptors may be an important mechanism for prompt regulation of body fluid volume, and ionic sensitivity of cardiovascular baroreceptors is possibly as important to body function as their pressure sensitivity.

DIANA L. KUNZE
WILLIAM R. SAUM
ARTHUR M. BROWN

Department of Physiology and Biophysics,
University of Texas Medical Branch,
Galveston, Texas 77550

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Interaction of DNA and liposomes as a model for membrane-mediated DNA damage

WE have described the antioxidant activity of calf thymus DNA on the oxidation of liposomal suspensions¹; we now report the damaging consequences to the biological activity of bacterial transforming DNA in such a system. Because DNA is associated with eukaryotic² and bacterial cell membranes³, the DNA-membrane interaction described here may be relevant to several pathological processes, including carcinogenesis⁴⁻⁷, ageing⁸⁻¹¹ and oxygen-dependent, radiation-induced cell lethality^{10,11}. In the presence of oxygen, the unsaturated fatty chains of membrane phospholipid undergo autooxidation¹². These autocatalytic free radical reactions, due to their chain nature, both amplify and propagate an initiating event occurring within the membrane, during which time many highly reactive and potentially damaging chemical species are formed¹³.

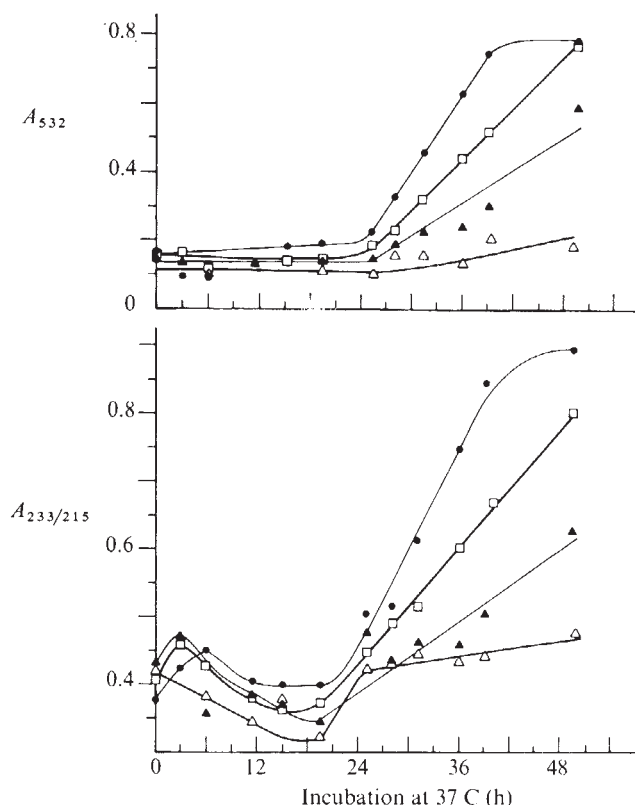
In view of the previous findings, we decided to investigate the possibility that retardation by DNA of the oxidative free radical reactions occurring within membrane phospholipids¹ would damage the DNA. Incubation mixtures of liposomes and *Bacillus subtilis* or calf thymus DNA were studied. Because liposomes are highly organised bilayers, they provide a model of *in vivo* membrane phospholipid configuration¹. The content of polyunsaturated fats was increased by enrichment with methyl arachidonate to enhance the rate of lipid oxidation in the bilayer system¹. Lipid oxidation was monitored colorimetrically by the thiobarbituric acid method (A_{532}) and spectroscopically by diene conjugation ($A_{233/215}$). DNA damage was assayed by measuring the reduction in transforming activity of *B. subtilis* BD 170 (thr 5 trp 2) cells. Figure 1 shows the effect of various concentrations of calf thymus DNA on the oxidation kinetics of methyl arachidonate-enriched phosphatidyl choline liposomes (PCMA)¹. None of the DNA concentrations studied increased the induction period, which in this experiment was approximately 21 h. The DNA did, however, retard the rate of lipid oxidation in a concentration-dependent manner. This and other results (ref. 14 and personal communication from K. M. Schaich and D. C. Borg) suggest that DNA interacts with the oxidising model membrane system as a free radical acceptor or trapping agent^{1,13}. Figure 2 shows the lipid oxidation kinetics of phosphatidylcholine (PC) and PCMA liposomal suspensions containing *B. subtilis* transforming DNA ($4.5 \mu\text{g ml}^{-1}$). The fatty chain profile, buoyant density characteristics and oxidation kinetics of these liposomes have been described before¹. The induction period for each suspension is the same, although the PCMA liposomes oxidise at a greater rate because of their higher degree of polyunsaturation. At the times indicated by the arrows in Fig. 2, DNA was isolated from each suspension and assayed for transforming activity (Table 1). DNA incubated in either sodium phosphate buffer or standard saline citrate (SSC) was stable during 68 h incubation, but DNA incubated with the oxidising liposomal suspensions lost its transforming activity in a manner dependent on the magnitude of lipid oxidation which has occurred in each suspension up to the time of sampling. In this respect, PCMA liposomes decreased the transforming activity of DNA by a factor of 2 during 16 h incubation, while the more slowly oxidising PC liposomes

caused no DNA damage. By 36 h incubation, PCMA liposomes had caused DNA transforming activity to decrease by a factor of 3,000 (the limit of the system), while PC liposomes had caused it to decrease by a factor of 8, again reflecting their lower oxidation rate relative to PCMA liposomes. After incubation with DNA for 68 h, PC liposomes also caused the largest detectable decrease in DNA transforming activity, by a factor of 3,000.

We also incubated *B. subtilis* DNA with PC and PCMA liposomes in SSC. During 68 h incubation, neither PC or PCMA underwent autooxidation, and accordingly DNA incubated in these non-oxidising systems retained its full transforming activity throughout the 68 h. Furthermore, fully saturated dipalmitoyl phosphatidyl choline liposomes suspended in sodium phosphate buffer underwent no autooxidation during 68 h incubation at 45 °C, and, correspondingly, *B. subtilis* DNA retained full transforming activity when incubated with these suspensions during that time. These controls show that non-oxidising membrane lipid *per se* has no effect on the transforming activity of DNA; for damage to occur, the lipids must be oxidising.

Taken together, these results indicate that oxidation of highly organised lipid bilayers can damage DNA. The species of oxidising lipid responsible for this damage are unknown but could be free radicals or reactive non-radical compounds. Free radicals, such as the $\cdot\text{OH}$ radical, which are produced during lipid autooxidation, have been shown to damage DNA¹⁵. The finding that DNA acts as a radical trapper in the model systems described before would support the hypothesis that DNA damage is caused, at least

Fig. 1 Oxidation kinetics of PCMA liposomal suspensions containing various concentrations of calf thymus DNA. PCMA liposomes were incubated alone or with various concentrations of dialysed calf thymus DNA in 0.05 M sodium phosphate buffer pH 7.5, as previously described¹. Total lipid concentration was 10 mM. Lipid oxidation was monitored by both A_{532} and $A_{233/215}$. Each point plotted represents the average of readings taken from two identically prepared liposomal incubation samples during a typical experiment. These results have been repeated three times. ●, PCMA; □, PCMA+DNA ($4.5 \mu\text{g ml}^{-1}$); ▲, PCMA+DNA ($45 \mu\text{g ml}^{-1}$); △, PCMA+DNA 225 ($\mu\text{g ml}^{-1}$).



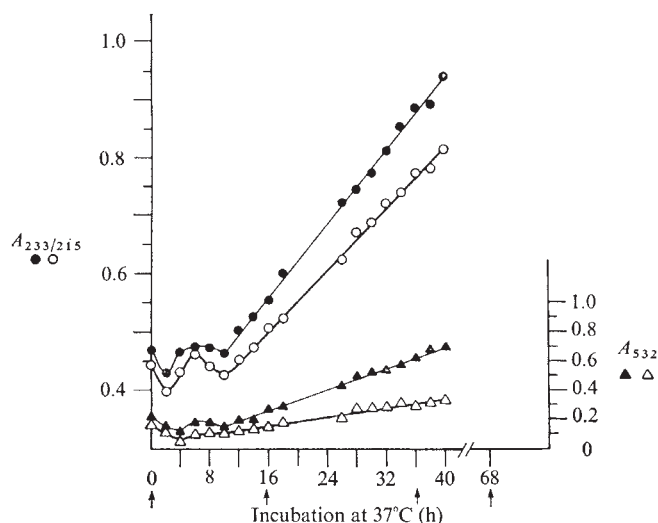


Fig. 2 Oxidation kinetics of PCMA and PC liposomal suspensions each containing *B. subtilis* DNA. PCMA and PC liposomes were incubated with *B. subtilis* ^3H -DNA ($4.5 \mu\text{g ml}^{-1}$) in 0.05 M sodium phosphate buffer pH 7.5, as described for PCMA liposomes and calf thymus DNA¹. Transforming ^3H -DNA (specific activity 2,000 d.p.m. μg^{-1}) was isolated from *B. subtilis* BD 204 (his B2 thy) (given by D. Dubnau) grown for four generations in SPI medium²⁹ containing L-histidine hydrochloride hydrate ($50 \mu\text{g ml}^{-1}$), thymidine ($1.5 \mu\text{g ml}^{-1}$) and $1.75 \mu\text{Ci } ^3\text{H}$ -thymidine ml^{-1} (19 Ci mmol^{-1}) by the Sepharose 4B procedure³⁰. Total lipid concentration was 10 mM in each suspension. Both A_{532} and $A_{233/215}$ were assayed to monitor lipid oxidation. Each point plotted is the average for four independent samples. The arrows indicate the times when samples were assayed for DNA transforming ability. ●, ▲, PCMA+DNA; ○, △, PC+DNA.

in part, by this radical attack. The carbonyl malondialdehyde is a typical non-radical, reactive compound formed during lipid peroxidation¹³. Malondialdehyde binds to DNA, decreasing its ability to serve as a template for RNA polymerase¹⁶. While unorganised emulsions of oxidising polyunsaturated fatty chains have been shown to damage DNA in conditions that enhance such carbonyl-DNA interaction (pH 4.6)¹⁷, the defined structural ordering in the membrane models used in the study reported here as well as the difference in pH between it and the previous work¹⁷, would make extrapolation difficult.

It has been suggested that membranes are the site of oxygen enhancement of radiation-induced cell lethality (the so-called "oxygen effect")^{10,11}. Supporting evidence includes

the effects of lipid-soluble inert gases¹⁸, the "time resolved O_2 effect"¹⁹, and the large O_2 effect on DNA-membrane complexes²⁰. In addition, ionising radiation initiates the oxidation of ordered micellar fatty chains with a G value of approximately 100 (ref. 21). Hence the type of membrane-mediated DNA damage described here is a possible mechanism of the O_2 effect, and the markedly higher solubility of oxygen in lipid, compared with water, makes this suggestion even more attractive²².

The relevance of this type of interaction in carcinogenesis is supported by the findings that both lipid peroxides and malondialdehyde are mutagenic^{23,24} and carcinogenic^{23,25}. Therefore, the components necessary to produce carcinogenic insult, that is O_2 and unsaturated fatty chains, are present in all cells in normal conditions. It has been proposed that lipid autooxidation is a continual process in living aerobic cells, and is restrained from entering the autocatalytic phase by enzymes and antioxidants⁸. Chemical and physical agents which enhance membrane free radical reactions may accelerate this process beyond the capabilities of those restraining systems and result in widespread membrane lipid oxidation and cell death^{13,26}.

Finally, it has been hypothesised that one cause of cellular ageing is membrane lipid oxidation-mediated damage to DNA and other macromolecules⁸. The studies described here demonstrate that oxidising membrane lipids impair the biological function of DNA and support this view.

The importance of the interaction of oxidising membrane lipids with DNA and/or other critical biomolecules in various pathological conditions can now be studied more directly using the unsaturated fatty acid-requiring auxotrophs isolated from *E. coli*²⁷ and Chinese hamster ovary²⁸ cell lines.

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DENNIS D. PIETRONIGRO*

W. BARRIE G. JONES

KATHY KALTY

HARRY B. DEMOPOULOS

Department of Pathology,
New York University Medical Center,
New York, New York 10016

Received 13 September 1976; accepted 1 March 1977.

*Present address: Department of Pharmacology, Yale University Medical School, New Haven, Ct 06510

Table 1 Transforming activity of *B. subtilis* DNA after incubation with PC or PCMA liposomes

Incubation time (h)	Sample incubated		
	DNA	DNA+PC	DNA+PCMA
0	3,250	3,320	2,850
16	2,690	3,105	1,471
36	3,190	427	0
68	3,030	0	0

At the times indicated by the arrows in Fig. 2 and listed above, a 0.5-ml sample was removed from each incubation sample, added to 10 ml cold absolute ethanol and centrifuged at 15,000 r.p.m. for 20 min at 4 °C. The resulting BD204 ^3H -DNA pellet was resuspended in 0.5 ml sodium phosphate buffer, pH 7.5. After 0.1 ml was counted to determine the ^3H -DNA concentrations, a volume corresponding to 0.45 μg of ^3H -DNA (0.15 ml) was assayed for ability to transform BD170 (thr 5 trp 2) to threonine independence²⁷. Transforming activity is the number of BD170 cells transformed by 0.45 μg of BD204 ^3H -DNA per 5×10^7 exposed cells. Incubation samples consisted of BD204 ^3H -DNA ($4.5 \mu\text{g ml}^{-1}$) alone in either 0.05 M sodium phosphate buffer, pH 7.5, or 0.015 M NaCl and 0.0015 M sodium citrate (SSC), pH 7.0, (DNA); or mixed suspensions of BD204 ^3H -DNA ($4.5 \mu\text{g ml}^{-1}$) with 10 mM PCMA (DNA+PCMA) or 10 mM PC (DNA+PC) in 0.05 M sodium phosphate buffer, pH 7.5, as shown in Fig. 2. Each value is the average for four independent samples. The standard deviation for each value is $\leq 10\%$.

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Gap filling during postreplication repair of DNA in recombination deficient *Escherichia coli*

POSTREPLICATION repair of DNA is one pathway for repair of damage inflicted on DNA by physical and chemical agents in the environment¹. Postreplication repair can involve error-prone repair of pyrimidine dimers in *Escherichia coli*², and as such may be particularly relevant to the understanding of the alteration of initial damage in DNA to a transformed or mutant DNA. The assignment of gene products to essential roles for completion of postreplication repair gap filling after ultraviolet light (UV) damage in *E. coli* cells³⁻⁷ has been complemented by consideration of their respective roles in error-prone repair of UV damage⁸⁻¹¹. We have used column chromatography, which separates double-stranded DNA containing single regions from completely double-stranded DNA, to observe the gap filling during postreplication repair of UV damage to DNA. In addition, analysis of *E. coli* *recA* or *lex* mutants demonstrated that the *recA*⁺ or the *lex*⁺ gene products must control steps exclusive of the initial gap filling process during postreplication repair.

DNA synthesised immediately after exposure to UV is discontinuous¹². Closure of these daughter strand gaps can be demonstrated by analysis of alkaline sucrose density gradient profiles. DNA increases in size (filling and joining) with reincubation. Alkaline sucrose centrifugation analysis of DNA size, however, does not differentiate the initial steps in gap filling from partially completed gap filling. In contrast, benzoylated naphthoylated DEAE-cellulose (BN-cellulose) chromatography is sensitive to the single-stranded regions in the double-stranded DNA structure and thus is suitable for analysis of the progress of gap filling. BN-cellulose chromatography was used successfully by Iyer and Rupp¹³ to measure the average gap size generated by DNA synthesis on UV-damaged parental DNA. We have used the same procedure.

E. coli cells were grown, exposed to UV and labelled with ³H-thymidine, and DNA was released and sheared as described by Iyer and Rupp¹³. Caffeine elutions from the BN-cellulose column of DNA from AB3063 cells with and without UV exposure were duplications of the procedure of Iyer and Rupp and gave the same results for the same strain. The work described here had an additional step, whereby some cells were reincubated in non-radioactive medium before caffeine elution. In addition, strains deficient in *recA*⁺ gene product were studied. The same procedure was used for a strain deficient in *lex*⁺ gene product and for its progenitor. The results shown here represent means of three to seven experiments with standard errors of the mean given for the caffeine elution.

In *E. coli*, the *recA*⁺ gene product controls postreplication repair. DNA from *recA* mutant cells that have been reincubated for 60 min after UV treatment does not increase to the size of DNA from unirradiated cells³. DNA from *E. coli* *lex* mutants demonstrates a slow increase in DNA size in the same conditions⁴. The results for postreplication repair observed indirectly by BN-cellulose chromatography in this study agreed with those of alkaline sucrose centrifugation studies: a protocol producing postreplication repair of daughter strand gaps in *recA*⁺ *lex*⁺ cells as indicated by gradient profile analysis resulted in a commensurate decrease in the number of daughter strand gaps indicated by BN-cellulose chromatography (Table 1). Analysis of BN-cellulose chromatography elution, however, demonstrated that *recA* or *lex* mutant strains, which are slow or unable to complete postreplication repair, were able to convert DNA with single-stranded regions to double-stranded DNA (Table 1). These experiments were repeated with additional *recA* strains including a *recA56recB21recC22* strain and isogenic *recA* and *recA*⁺ strains obtained from Dr S. D. Barbour (not shown), and the results indicated that wild type cells and mutants could convert equal amounts of gapped DNA to double-stranded DNA. Thus, while *recA*⁺ and *lex*⁺ gene products are essential for normal levels of postreplication repair, mutants demonstrate extensive conversion of UV-induced postreplication daughter strand gaps to double-stranded DNA.

Howard-Flanders¹ and Hanawalt¹⁴ have reviewed the possible mechanisms of postreplication gap filling. They discussed the roles, relative to the gap filling process, of *de novo* DNA synthesis, DNA strand exchange and the process by which gap filling is initiated. Our results support the hypothesis that the *lex*⁺ and *recA*⁺ gene products in wild type cells are essential for postreplication repair gap closure after initiation of gap filling and/or the first steps thereof. Essentially, the function of the error-prone repair *recA*⁺ or *lex*⁺ gene product seems to be the filling of the minority of the gap.

An alternate explanation for the gap filling observed in *E. coli* *recA* or *lex* mutants could involve preferential degradation of the single-stranded parental DNA forming the gap backbone. Although the stability of the parental DNA was not measured in the experiments shown here, degradation might be below detectable levels even if the parental DNA were labelled. Nevertheless, such degradation would precipitously decrease the size of parental DNA as measured with alkaline sucrose gradient analysis during post-UV reincubation of *recA* mutants. This has not been observed in gradient analysis in this laboratory. More importantly, in the *recArecBrecC* mutants, with greatly reduced degradation ability, loss of daughter strand gaps was observed as with the *recA* mutant (not shown).

An alternative hypothesis is that gap filling represents the function of proteins produced by leaky mutants. If, however, the *recA*⁺ gene product were responsible for the control of all the steps

Table 1 Step elution from BN-cellulose of ³H counts from preparations of irradiated or unirradiated *E. coli*

<i>E. coli</i> strain	Strain ref.	Pertinent genotype	UV irradiation 6.0 J m ⁻²	Reincubation 50 min	% Recovered counts eluting with	
					No caffeine	2% Caffeine
(K) AB3063	(13)	<i>uvrA</i> EndoI	—	—	95	5 ± 1
			+	—	40	60 ± 5
			+	+	88	12 ± 3
(K) AB2487	(3)	<i>recA13</i>	—	—	87	13 ± 1
			+	—	60	40 ± 6
			+	+	84	16 ± 1
(B) WP2	(9)		—	—	93	7 ± 1
			+	—	67	33 ± 2
			+	+	95	5 ± 2
(B) WP5	(9)	<i>lex</i>	—	—	86	14 ± 6
			+	—	70	30 ± 5
			+	+	92	8 ± 2

in gap filling in leaky mutants, a 60 min reincubation should permit significant gap closure, measurable on alkaline sucrose gradients, which is not observed³.

Studies by Witkin⁹ and others suggest that some *E. coli recA* mutant cells can survive low doses of UV but are differentially repaired with less error than *recA*⁺ survivors. Presumably some completed gap filling occurs after a 24 h reincubation⁹. The identity of proteins responsible for this completion of gap filling or final closure and the fate of the pyrimidine dimer during error-prone repair are now under investigation in this laboratory.

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ROBERT CAREY JOHNSON

Department of Basic and Clinical
Immunology and Microbiology,
Medical University of South Carolina,
Charleston, South Carolina 29401

Received 17 December 1976; accepted 1 March 1977.

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Repair of DNA damaged by alkylating carcinogens is defective in xeroderma pigmentosum-derived fibroblasts

THE human hereditary skin disease xeroderma pigmentosum (XP) is characterised by extreme sensitivity to sunlight and a high incidence of sunlight-induced skin cancer. These symptoms seem to be correlated with a defect in the excision repair of ultraviolet-induced dimers¹. Repair of DNA damage caused by chemical carcinogens belonging to the group of monofunctional alkylating agents was thought to be normal in XP cells, however, because after treatment with these agents the amounts of repair replication^{2,3} and the numbers of chromosome aberrations⁴ were similar in normal and XP cells. Contrary to this indirect evidence, it is reported here that direct measurement of the amounts of two products formed by alkylating carcinogens in the DNA and of the rate at which they are eliminated indicates that XP cells have a defect in this type of repair also.

XP-derived fibroblasts and normal human fibroblasts were treated with the potent carcinogens *N*-methyl-*N*-nitrosourea (MNU) and *N*-ethyl-*N*-nitrosourea (ENU). From the alkylation products formed, only the amounts of 7-alkylguanine and *O*⁶-alkylguanine were measured—7-alkylguanine because it is a major product and indicates the overall reactivity of the carcinogen with nucleophilic centres in the DNA and *O*⁶-alkylguanine because it seems to be critical for the mutagenic and carcinogenic action of alkylating agents^{5–9}. *O*⁶-alkylguanine has been shown to cause mis-coding and anomalous base pairing⁵, and the relative amounts of this base that are produced by different agents seem to correspond to their carcinogenic potency⁶. In addition, the inability of an organ to excise *O*⁶-alkylguanine enzymatically from its DNA correlates with the organ-specific carcinogenic action of alkylating agents^{7–9}.

Human fibroblasts from a normal donor (GM637) and from an XP patient of complementation group A (XP12RO) were treated with 8.7 $\mu\text{g ml}^{-1}$ ³H-MNU or 100 $\mu\text{g ml}^{-1}$ 1-¹⁴C-ENU for 45 min. The DNA was isolated 0, 24 and 48 h after the treatment and, after DNA hydrolysis, the purine bases were separated by Sephadex G10 chromatography (see Table 1 and ref. 10). The amount of each alkylguanine was determined from the radioactivity eluted in the position of the appropriate marker and was expressed as a fraction of the total guanine.

Normal and XP cells had about the same amounts of alkylated guanine immediately after treatment with an alkylating agent (Table 1), and the relative yields of *O*⁶-alkylguanine and 7-alkylguanine corresponded to those found *in vivo* in the rat^{10,11} and *in vitro* after reaction of DNA with MNU or ENU (refs 6, 10). The loss of 7-alkylguanine from DNA was similar in both cell lines, but there was a significant difference in the loss of *O*⁶-alkylguanine. Whereas in normal cells *O*⁶-alkylguanine was reduced to 28% of the initial amount 48 h after MNU treatment and to 9% 48 h after ENU treatment, there were still 63% (MNU) and 70% (ENU) of the initial *O*⁶-alkylguanine in XP cells at this time.

These reductions of alkylguanines partly reflect dilution by cell proliferation because the amount of alkylguanine is expressed as a fraction of the total guanine. To exclude this dilution effect and to eliminate differences in the rate of cell proliferation between cell lines, *O*⁶-alkylguanine was expressed as a fraction of 7-alkylguanine (Fig. 1). In normal cells this ratio decreased to one-third of the initial value 48 h after treatment with ENU and stayed constant after treatment with MNU, indicating the *O*⁶-alkylguanine was lost from the DNA as fast as or faster than 7-alkylguanine. In XP cells, however, the ratio of the two agents about doubled during the 48 h for both ENU- and MNU-treated cells, which means that 7-alkylguanine was lost much faster than *O*⁶-alkylguanine in these cells.

The reason for the similar rates of loss of 7-alkylguanine in normal and XP cells but the very different rates of loss of *O*⁶-alkylguanine may involve different mechanisms for removing the two bases. Alkylation of guanine at the N7 position labilises the *N*-glycosidic bond and leads to non-enzymic depurination of the base¹². A mechanism of 7-alkylguanine loss based on spontaneous depurination and dilution by cell proliferation predicts that similar rates of loss would occur in cells that have similar division rates. This was the case in normal and XP cells (Table 1), although enzymic excision cannot be excluded. *O*⁶-alkyl-

Fig. 1 Ratio of *O*⁶-alkylguanine to 7-alkylguanine in the DNA of normal human fibroblasts (---) and XP fibroblasts (—) after treatment with methyl nitrosourea (○) or ethyl nitrosourea (▲) as a function of time.

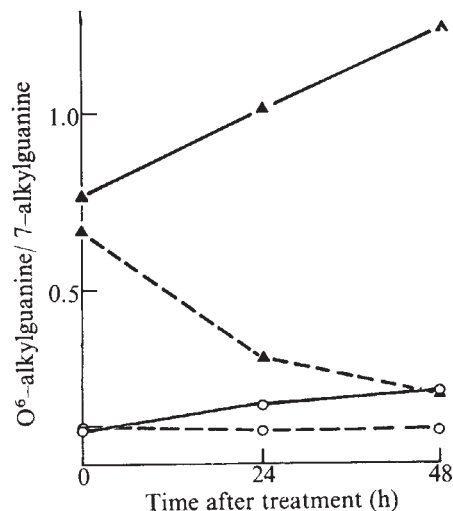


Table 1 Amount of alkylated guanine in the DNA of normal and xeroderma pigmentosum fibroblasts after treatment of the cells with ^3H -methylnitrosourea or $1\text{-}^{14}\text{C}$ -ethylnitrosourea as a function of time

Cell type	Time after treatment (h)	^3H -methylnitrosourea (8.5×10^{-6} M)				$1\text{-}^{14}\text{C}$ -ethylnitrosourea (8.5×10^{-4} M)			
		7-Methyl-guanine guanine ($\times 10^6$)	% of 0 h value	O^6 -Methyl-guanine guanine ($\times 10^6$)	% of 0 h value	7-Ethyl-guanine guanine ($\times 10^6$)	% of 0 h value	O^6 -Ethyl-guanine guanine ($\times 10^6$)	% of 0 h value
GM637	0	181	—	19	—	31	—	21	—
	24	106	59	10	53	17	55	5	24
	48	51	28	5	28	9	30	2	9
XP12RO	0	169	—	16	—	30	—	23	—
	24	78	46	14	88	16	53	16	70
	48	48	28	10	63	13	43	16	70

Cell lines, which had been transformed by SV40 virus, were chosen because of their high rate of cell division. Previous studies have demonstrated that transformation does not alter DNA repair characteristics of human fibroblasts^{21,22}. The cells were grown in Eagle's minimum essential medium with 10% calf serum in 1-gallon roller bottles. For one alkylation experiment cells from four confluent cultures were trypsinised and resuspended in 25 ml of serum-free Eagle's minimum essential medium in a Spinner flask and stirred at 37 °C at a speed that kept cells in suspension. The alkylating agent was then added—8.7 $\mu\text{g ml}^{-1}$ ^3H -MNU (123.2 Ci mol⁻¹, 0.87 mg ml⁻¹ in ethanol) or 100 $\mu\text{g ml}^{-1}$ $1\text{-}^{14}\text{C}$ -ENU (4.98 Ci mol⁻¹, dissolved just before use in citric acid-disodium phosphate buffer, pH 6)—and the cells were incubated for 45 min. DNA from one-third of the cells was isolated immediately. The rest of the cells were put back into roller bottles and grown for 24 or 48 h before collection by trypsinisation. Cells were lysed in 0.1% sodium dodecylsulphate in SSC (0.15 M sodium chloride + 0.015 sodium citrate) and then incubated first with RNase (final concentration 0.1 mg ml⁻¹) for 1 h and then with pronase (final concentration 1 mg ml⁻¹) for 2 h at 37 °C. The DNA was precipitated by 2 volumes of ethanol and hydrolysed in 0.1 N HCl (37 °C, 20 h); the purine bases were separated by chromatography on a Sephadex G10 column (80 $\times$ 1.5 cm) eluted with 0.05 M ammonium formate (pH 6.8). The amount of each alkylated guanine was calculated from the specific radioactivity of the given alkylating agent and the integral radioactivity of the chromatographic peak corresponding to a marker for the appropriate alkylated base, and was expressed as a fraction of the total guanine in the isolated DNA, which was determined from the absorbance of the guanine fractions from the column eluates. For details see ref. 10.

guanine, on the other hand, is chemically stable in DNA^{10,13} and must be enzymically excised. Because the XP cells under investigation lost much less O^6 -alkylguanine than normal cells did, this loss might have been completely due to dilution by proliferation rather than to excision.

Although the doses of MNU and ENU used in this experiment produced almost the same amount of O^6 -alkylguanine, the observed excision from the DNA of normal cells was much faster after ENU treatment than after MNU treatment (Table 1, Fig. 1). This might imply that the ethylated derivative is excised more efficiently. But, MNU also produced much higher overall alkylation in the cell, the alkylation at the N7 position of guanine being, at the doses used, about six times higher than with ENU, so a similarly higher reaction with proteins can be expected. Thus, a reasonable explanation for the slower excision of O^6 -methylguanine is that enzymes of the repair system are partially inactivated by the MNU dose used.

Though it has been shown that O^6 -alkylguanine is excised enzymically in mammalian cells, it is not yet known if the enzyme is of the nucleotidase type or of the N -glycosidase type¹⁴. The enzyme that excises O^6 -alkylguanine in *Escherichia coli*, endonuclease II¹⁵, seems to be an N -glycosidase that works in conjunction with an apurinic endonuclease¹⁶. If the same mechanism is at work in mammalian cells, then XP cells of complementation groups A and D may have a defect in the entire repair process, because it has already been shown that there is a defect in their apurinic endonuclease activity¹⁷.

This type of repair seems to have an important role in carcinogenesis. It has been shown that the rate at which O^6 -alkylguanine is excised varies greatly in different rat tissues, being fastest in liver, intermediate in kidney, and very slow in brain⁷⁻⁹. As MNU and ENU are both nervous-system-specific carcinogens in the rat¹⁸, the inability of the brain to excise this potentially mutagenic damage correlates with the organ-specific carcinogenic action of these agents. The inability of XP-derived fibroblasts to excise this damage might not be representative of all XP-derived cell types, although it seems unlikely that the inheritable change connected with this disease would be expressed in only one cell type.

It has recently been reported that, unlike the number of chromosome aberrations, the frequency of sister chromatid exchanges is much higher in XP cells than in normal cells

after treatment with alkylating agents¹⁹. This raises the question of whether there is a causal relation between the unexcised O^6 -alkylguanine and the increased sister chromatid exchange.

Because measurements of repair replication by isopycnic analysis and by autoradiography after treatment with alkylating agents do not show a difference in repair by normal and XP cells^{2,3}, these techniques do not seem applicable when various types of damage occur. It especially does not seem to be an accurate measure of excision, because the main product, 7-alkylguanine, depurinates spontaneously. Also, although almost no repair is detectable by this technique 20 h after ENU treatment of normal cells²⁰, the work described here shows that more than 50% of the alkylated bases are still present in their DNA.

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REGINE GOTH-GOLDSTEIN*

Laboratory of Radiobiology,
University of California,
San Francisco, California 94143

Received 27 December 1976; accepted 8 March 1977.

*Present address: Donner Laboratory, University of California, Berkeley, California 94720.

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reviews

Algal genetics

Lynn Margulis

The Genetics of Algae. Edited by Ralph A. Lewin. Pp. viii + 360. (Blackwell Scientific: Oxford and London, 1976.) £11.50.

THIS book is a beautifully edited and profound review of the state of genetics of algae, which, as Lewin says, "are more of a guild than a clan, united more by common aptitude than by common ancestry". Although all algae have been taken to be within the province of the work, in fact there is very little serious genetics on organisms other than the green algal genus *Chlamydomonas*.

There are thirteen separately authored sections in the book. These include reviews of the recently established field of the genetics of blue green algae, the formal (mainly nuclear, mendelian) genetics of *Chlamydomonas reinhardtii*, genetic determinants of flagella phenotype in *C. reinhardtii* (a frustrating subject to study since flagellar alterations in these organisms which mate 'flagella first', tend to impair the frequency of viable crosses) and a short chapter on the genetics of cell wall formation in *C. reinhardtii*. The cell wall summary not only indicates how useful mutational analysis ultimately may be in the deciphering of the complexities, chemical and ultrastructural of cell wall morphogenesis, but indicates that there may be still another enigmatic "non-mendelian genetic system" in this chlorophyte.

C. reinhardtii, of course, is the star actor in any eukaryotic cell genetics drama and this is aptly indicated in the two chapters by Gillham, Boynton and their colleagues on plastid inheritance and genetic control of ribosomal biogenesis. The relationship of the three genetic systems (nuclear, plastid and mitochondrial) has nowhere been better investigated than in this rapidly growing isogamous organism, which may be mated at will, completes its life cycle in about a week and for which gene dosage and genotype-phenotype relationships can be studied since stable diploids can be made.

Information concerning the genetic systems of *C. moewusii* and *C. eugametos*—two other species of *Chlamydomonas* rivalling *C. reinhardtii* for centre stage—is reviewed. Since

stable heterokaryons can be made in *C. moewusii*, complementation can be studied in this organism. The reason why 2 amino-3 butanoic acid induces both gene mutation and chromosomal aberration in *C. eugametos* is still obscure. No such effect has been seen in *C. reinhardtii*. Other differences between the three species have been noted as well, providing internal tests for the possible generalisation of observations made on *C. reinhardtii*.

Although an exciting comprehensive, and up-to-date treatise, this book makes us acutely aware of how limited our genetic knowledge is concerning the algae as a group. There literally is no genetic literature as such on the red algae, the brown algae, the diatoms, the dinoflagellates, the chrysophytes, the prasinophytes, the euglenids, the haptophytes, and so forth. Although there is some scattered information concerning the mating and differentiating systems in *Volvox*, conjugating green algae, 'multicellular marine algae' and *Acetabularia* in chapters on the genetics of the Volvocales, Zygnematales, and Charophyta, respectively, even these sexual green algae have managed to hold on to their genetic secrets.

The classical techniques of controlling the life-cycle of marked genetic stocks, crossing individuals of opposite mating types and measuring the distribution of traits in offspring of these crosses are simply not available in the vast majority of algae for several different reasons. For example, the genetics of an organism such as *Euglena gracilis*, a flagellate which has yielded significant information concerning mitochondria and chloroplast inheritance and development, is impossible to study. Although chloroplast development and differentiation can be induced, these fast growing single cells simply refuse to mate. Thus, all of these chapters referred to above are forced to deal with "genetically related" phenomena (for example, hybrids produced by grafting in *Acetabularia*, nucleic acids of organelles).

Furthermore, the book is overwhelmingly concerned with the eukaryotic green algae: the Chlorophyta. As for the Cyanophyta or blue greens, they are a world apart. True to their popular new name (Cyanobacteria) they are

only rudimentarily sexual, anything at all about genetics is known in very few species. In general, they rely on proven prokaryotic mechanisms for gene transfer—transformation and transduction—in so far as any occurs. One new realisation is the nature of the circular genetic map as deduced from mutagenesis studies in synchronous cultures of *Anacystis nidulans*, a coccoid blue green: it really resembles in overall features, the genetic map of *Escherichia coli*.

Some idiosyncratic but profound algal genetic anecdotes come to light in this well illustrated and produced book. For example: in some blue greens recombination seems to be regularly accompanied by mutation; in *Ulva* the chromosomes are apparently composed of four rather than two DNA strands, and in *C. eugametos* a specific mutator gene reverses mutations at two nicotinamide loci.

Probably the most exciting advances concern the unravelling of biochemical interactions between the three classes of genetic organelles in *C. reinhardtii*: nucleus, mitochondria and plastids. Indeed sex in organelles exists and genetic maps have been made of a cytoplasmic genetic system. (Presumably these maps are of the chloroplast linkage groups: recombination of plastid DNA has been discovered.) There is even some evidence that a single mutation may alter both chloroplast and mitochondrial ribosomes simultaneously. Several chloroplast ribosomal proteins are apparently synthesised on cytoplasmic ribosomes even though the chloroplast DNA probably codes for all the chloroplast RNA.

The book is full of such well explained surprising genetic phenomena, and is highly recommended for advanced genetic and phycology courses and the professional reader. *The Genetics of Algae* is indispensable to those interested in genetic systems of the 'lower eukaryotes'. The book is well written, comprehensive and comprehensible. If more work on the other fascinating algal groups has not been reported here, it is because it has not yet been done. □

Lynn Margulis, Professor in the Department of Biology at Boston University, is on leave as Sherman Fairchild Distinguished Scholar at the California Institute of Technology, Pasadena.

Aggregation chimaeras

Mammalian Chimaeras. By A. McLaren. Pp. 154. (Cambridge University: Cambridge, London and New York, 1976.) £8.

It might be said that the laboratory mouse is the *Escherichia coli* of modern mammalian biology, since great things are expected of this species. Mouse genetics is one field in which the Woman's Liberation Movement is already passé, for the field has long been dominated by a group of outstanding ladies. Dr McLaren is certainly one of them. Her little book, as she lovingly calls it, certainly shows her intelligence and breadth of understanding.

Characteristically feminine attentions to details are most pleasing. The subjects are mostly what have variously been called allophenic or tetraparental mice produced by the fusion of two blastocysts, but Dr McLaren prefers to call them aggregation chimaeras. Injection chimaeras refer to those that are produced by the injection of one or more cells into a blastocyst of a different genotype as practiced by Richard Gardner. These animals are very useful for analysis of clonal differentiation of embryonic cells. Degrees of intermingling that inevitably occur between neighbouring clones can also be assessed. They are also ideally suited to distinguish genetic effects that are mediated through humoral factors from those that are

exclusively intracellular. The wealth of information accumulated by Tar-kowski, Minz, McLaren, and by a number of others, is presented in a very orderly manner and in sufficient detail.

Inevitably, a few very important pieces of information apparently failed to make the deadline, but who can blame the author for this. For example, M. F. Lyon's recent demonstration that totally androgen-insensitive *Tfm/Y* male germ cells in the body of chimaeric male mice can mature and differentiate to functional spermatozoa was nearly a fatal blow to those who believed in the androgen dependence of male germ cells. Similarly, the discussion on the great preponderance of males among XY/XX chimaeric mice would have become more meaningful if more recent findings on H-Y antigen's testis-organising functions were introduced.

With regard to immunology, another recent finding that Ia antigens, controlled by at least two immune response (I_r) loci, are very strongly expressed on B cells, but only very weakly on most T cells, would surely have modified Dr McLaren's discussion of findings on chimaeric mice between high responder and low responder strains.

I believe that almost every problem-solving biologist who deals with higher vertebrates would find this book instructive and informative.

Susumu Ohno

Susumu Ohno is Chairman of the Division of Biology, City of Hope National Medical Center, Duarte, California.

Practical problems of the visual world

Vision and Acquisition: Fundamentals of Human Visual Performance, Environmental Influences and Applications in Instrumental Optics. By Ian Overington. Pp. xix 380. (Pentech: London; Crane, Russak: New York, 1976.) £14.

This is an unusual and useful book. Its main value is that it focuses attention on the problem of how a human detects and recognises a target in adverse conditions.

Although much of the work described was undertaken for military purposes, many of the results are pertinent to civilian life—for example, the flying of modern high speed aircraft poses many human problems particularly in visual performance. But even at the lower speed of driving a car many accidents are believed to occur because of a failure in visual perception or recognition. This is

particularly true in motorway driving in poor visibility, often resulting in a tragic loss of life and injury.

The book is particularly valuable in that it contains many references to special reports that most visual scientists would not come across. There are a few errors particularly when the physiology of vision is discussed, but none of these are of a misleading nature.

I hope that this book will encourage some laboratory bound visionaries to look out of the window and consider the practical problems that the visual world presents. Conversely, it may also encourage engineers to pay more attention to modern visual physiology and possibly even to seek the advice of visual scientists more frequently.

The book is very neatly presented and illustrated, and bears the high price of today's publication costs.

F. W. Campbell

F. W. Campbell is Reader in the Department of Physiology at the University of Cambridge, UK.

Virology symposia

Animal Virology. (ICN-UCLA Symposia on Molecular and Cellular Biology. Vol. IV. Edited by D. Baltimore, A. S. Huang and C. F. Fox. Pp. xi+824. Academic: New York and London, 1976.) \$29.50; £18.

THE ICN-UCLA Symposia on Molecular and Cellular Biology held each spring at skiing resorts in California or Colorado provide a pleasant and useful forum for the subjects discussed. Baltimore, Huang and Fox organised what seems to have been a most interesting meeting on animal viruses, that even included a session on plant viruses. Much of the emphasis was on viruses known to be oncogenic, including an interesting session on cell transformation and differentiation, but there were also sessions on the molecular organisation of negative-strand RNA viruses, on viral messenger RNA synthesis, and on viral protein synthesis. Thus, as the organisers say in the preface (as editors of the published proceedings), "this meeting considered broad issues and recent advances in animal virology, and treated tumor viruses in the context of the range of animal viruses rather than as a separate problem."

In my opinion this volume should never have been published. True, it is one of the better volumes of its kind, but I deplore the practice of routine publication of symposia proceedings. It clutters up the literature and overburdens library budgets. Perhaps some of these volumes are profitable to publishing companies: they are not profitable to scientific endeavour. The only purpose it serves is to amplify the contributors' publication lists. The ICN-UCLA symposia are published by typescript photo-reproduction methods and appear about 6 months after the meetings are held. Most of the papers in the volume under review had by that time been printed with essentially identical data in academic journals as proper publications. Those that have not, may have been disapproved by reviewers and editors. The symposia proceedings are not subject to review and there is no distinction between invited and proffered papers.

Scientific organisers of symposia are usually apologetic on requesting manuscripts for publication, explaining that the financial sponsors demand them. The sponsors should realise that the meeting is important, not the publication, and there are other ways of making the sponsorship known. It is time that scientists declined to present talks on current research at symposia if publication is obligatory.

Robin Weiss

Robin Weiss is a member of staff at the Imperial Cancer Research Fund Laboratories, London, UK.

Bioengineering

Applied Biochemistry and Bioengineering. Vol. 1: Immobilized Enzyme Principles. Edited by L. B. Wingard, Jr, E. Katchalski-Katzir and L. Goldstein. Pp. xi+364. (Academic: New York and London, 1976.) \$29.50; £20.95.

RECENTLY there has been a spate of books on bioengineering in general and enzyme technology in particular. Many are collections of unrelated articles of very variable quality. Therefore it is pleasant to note that this first volume of a new series has a coherent theme which is well treated. The editors do have a particular reason to organise the volume well. Wingard has done much to stimulate interest in enzyme technology, and Katchalski-Katzir and Goldstein were pioneers in the fundamental studies of immobilised enzymes. Appropriately the latter two editors begin the book with a concise survey of developments in the field which serves to introduce subsequent contributors. Goldstein is also co-author (with Manecke) for the second chapter on the chemistry of enzyme immobilisation. The systematic treatment of the available methods with comment on their advantages and disadvantages will be welcomed by anyone who has to make a selection from the bewildering number now available.

Engasser and Horvath deal with diffusion and kinetics of immobilised enzymes.

Synaptic connections

Neuronal Recognition. By S. H. Barondes. Pp. xvi+367. (Plenum: New York and London, 1976.) \$33.

THIS collection of eleven essays is concerned with the problem of how neurones establish synaptic connections with certain targets and not with others. As a result of studies on the developing and regenerating nervous system, we now have a reasonable picture of the degree of discrimination in several situations, but not of the underlying cellular and molecular mechanisms. The essays vary from relatively general reviews to quite detailed accounts of work from the authors' laboratories. Together, they provide a good overall view of approaches to the problem.

The existence of specificity, as well as its extent and possible basis, is considered for the retinotectal system (Jacobson), for the neuromuscular junction (Fambrough), for sprouting in the central nervous system (Cotman and Lynch), and for synaptogenesis in tissue culture (Bunge). Morphological information about early events in synapse formation is considered by Pfenninger and Rees, and the current unsatisfactory state of biochemical methods for purifying and

They draw heavily on the results of their collaborative studies in dealing, for example, with the effect of external diffusion limitation on the observed behaviour of enzymes immobilised in porous particles or membranes.

The review of the design and analysis of immobilised enzyme flow reactors by Vieth *et al.* is well documented but the reviewer would arrive at some different conclusions. The authors state that 'batch reactors have limited potential in industrial immobilised-enzyme catalysis.' Most of the semisynthetic penicillins produced industrially in Europe and a growing proportion elsewhere are made by means of batch conversion using immobilised enzyme.

The industrial application of immobilised enzymes and immobilised microbial cells are described with technical and economic details by Chibata and Tosa, whose Japanese company was the first to announce the use of both types of catalyst in modern industrial processes. It is interesting that glucose isomerisation, which originated in Japan but was developed as the largest-scale current use of immobilised cells or enzymes in the USA, is not dealt with in any detail, presumably because it does not have the same economic importance in Japan.

P. Dunnill

P. Dunnill is Lecturer in Biochemical Engineering and co-leader of the enzyme and protein technology group in the Department of Chemical and Biochemical Engineering at University College, London, UK.

fractionating neural plasma membranes is critically reviewed by Morgan and Gombos.

The second half of the book is concerned with attempts to assay and identify molecules involved in cell recognition in various situations. Although there is, as yet, no direct evidence on the relevance of these studies to synapse formation, they are clearly of great interest to students of specificity. Three of these essays are concerned with investigations of cellular aggregation and adhesion in the retinal and retinotectal systems (Moscona; Roth and Marchase; Merrell, Gottlieb and Glaser). The morphogenetic role of extracellular glycosaminoglycans is considered by Toole, and Barondes and Rosen discuss cell surface lectins and cell recognition in slime moulds.

Most of the essays are thoughtful, well organised and often helpfully illustrated. Neurobiology sometimes seems impenetrable to outsiders, but I would strongly recommend this volume to interested investigators in other areas of cellular and molecular biology, as well as to graduate students and specialists in the field.

Jeremy Brockes

Jeremy Brockes is a member of the MRC Neuroimmunology Project at the Department of Zoology, University College, London, UK.

Fourier transform NMR spectroscopy

Fourier Transform NMR Spectroscopy. By D. Shaw. Pp. xvi+357. (Elsevier Scientific: Amsterdam, Oxford and New York, 1976.) Dfl. 129; \$49.75.

FOR a great deal of chemical structural and diagnostic work using proton nuclear magnetic resonance (NMR), a simple continuous wave (CW) spectrometer is entirely adequate, but in cases involving very small molar quantities of sample or for measurements on other nuclei, Fourier transform (FT) spectrometers are needed. The CW spectrometer scans slowly across the spectrum, using a continuous monochromatic radio frequency to stimulate each resonance in turn. In the FT spectrometer, a brief but strong pulse of radio frequency is applied to the sample so as to stimulate all the resonances simultaneously. The response of the nuclear spins after the pulse is collected, and because the components of all the resonances are mixed up together (and in the time domain) a computer is needed to disentangle them and produce the more familiar spectrum (in the frequency domain). Because all the resonances are stimulated simultaneously, there is a substantial gain of signal-to-noise for complex spectra, and because the method uses pulsed radio frequencies, relaxation times are more easily measured than with the CW method.

Dr Shaw has produced an excellent handbook for those spectroscopists who are extending their activities from the CW to FT methods. In chapter 3 the mathematical principles are outlined, not in an attempt to provide rigorous accounts of the theorems, but to ensure that the physical principles are clear and the equations used can be understood. Chapters 4 and 5 give a clear description of the various ways of exciting NMR spectra and their relative advantages; and chapters 6 and 7 explain in some detail the experimental methods available, the equipment required, how it works, and the ways in which it may be used. These chapters form the core of the book and will be of great value to all those who need to use FT spectrometers.

Chapters 1 and 2 give a brief introduction to the principles of NMR spectroscopy; and chapters 8, 9, 10 summarise many of the ways in which NMR spectroscopy can be applied to chemical problems and how the measurable parameters can be obtained. These chapters cover a lot of ground and are useful mainly as a convenient outline and reminder to readers already acquainted with the subject.

R. E. Richards

R. E. Richards is Warden of Merton College, Oxford, UK.

Machiavellian weed

Wild Oats in World Agriculture: An Interpretative Review of World Literature. Edited by D. P. Jones. Pp. xi+296. (Agricultural Research Council: London, 1976.) £3.

It is unusual, if not unique, for a weed to achieve the distinction of having such a comprehensive publication devoted to itself, an accolade most often accorded to a crop species. The impetus for a review at this time has been dictated primarily by the requirements of agriculture for greater understanding of the biology and control of this pernicious species. It is perhaps Joan Thurston at Rothamsted, however, who must be acknowledged as having foreseen in the post-war period the emergence of this species as a primary constraint to the cereal producer and initiated much needed research and the collection of material from throughout the world, on which so much of the information on distribution of *Avena fatua* is based.

The significant contribution to the systematic appraisal of the biology and agronomy of wild oats by the Weed Research Organisation in the last decade is reflected in the authorship of this book. A leavening of the text, however, with some contribution from outside the UK, would have been useful in consolidating the purported world aspect of the review. Research in Canada with respect to the fundamental mechanisms of seed dormancy and at a more general agronomic level has been considerable. The whole nature of the problem in North America, with its extensive low input cereal production system, stands in stark contrast to the situation in Western Europe: the role of Government and statutory controls could also have been treated in a somewhat wider perspective.

A wealth of information has been gathered together in this volume. In this topic, there undoubtedly exists the basis of a classic contribution, not just to agriculture or botany but of relevance to many areas within the broad biological discipline. There are so many elements in the biology of the wild oat of wide and general significance—a weed par excellence and one whose resources and versatility, strengths and weaknesses, have been so well documented to exemplify the myriad facets of a successful weed—that it is to be hoped that this volume will achieve a circulation outside that of the specialist agriculturalist for whom it was mainly intended. The mechanisms of seed

dormancy and seed dissemination, the population dynamics in the context of agricultural practice, the niceties of selective herbicide usage which now allow a distinction to be drawn between the 'wild' and cultivated varieties of oats, are examples of situations of wide biological relevance.

My prime reservation is that the authors have aimed this work at a specialist readership, whereas the subject has a great deal of popular appeal and that the fascinations of this Machiavellian weed have been subjugated, if not totally submerged, by the bulk of references and verbiage.

On a more specific level, although the chapters follow a logical sequence there are incongruous aspects to the

section and subsection headings in certain chapters. A degree of unnecessary duplication of material between topics is also to be found although, with the exception of minor omissions in pagination, the difficult task of adequate cross referencing has been well accomplished.

In all, this volume stands as a novel concept within, and a valuable contribution to, the field of the biology and control of weeds, and at such a modest price represents exceptional value.

Alan D. Courtney

Alan Courtney is Lecturer in the Department of Agricultural Botany at the Queen's University, Belfast, Northern Ireland.

Laser applications

CO₂ Lasers: Effects and Applications. By W. W. Duley. Pp. xiii+427. (Academic: New York, San Francisco and London, 1976.) \$31; £18.40.

THE preface to this book is more informative than its title: the volume outlines a very wide range of laser applications in physics, chemistry and engineering, with the CO₂ laser receiving special rather than exclusive attention. In places the treatment is distinctly pragmatic. For example, in the forty or so pages dealing specifically with CO₂ laser devices, optimisation of optical cavity transmission is treated entirely experimentally, with no discussion of Rigrod's formulation. A second example is provided in the surprisingly brief treatment of unstable optical resonators, where there is no interpretation of the specific experiments cited.

Readers may feel that chapter 3 redeems itself with the very detailed information provided on infrared detectors, windows, modulators and mirrors, although diamond turning techniques receive no mention. The first half of the book concludes with selected solutions to the heat-conduction equation, a subject previously, exhaustively and authoritatively treated by Carslaw and Jaeger. Moving heat sources, liquid phases and vaporisation do, however, provide interesting variations to this theme, and are required for a proper understanding of surface heat treatment and welding; here, an important reference (Swift-Hook and Glick, 1973) has been inadvertently omitted in the references.

An interesting survey of drilling by argon, ruby, Nd-YAG and CO₂ lasers is followed, in chapter 6, by a somewhat dated discussion of welding and machining. In particular, Table 6.5

contains several errors and omits any specification of spatial mode quality of the laser, or of focal conditions—factors which may account for some of the relatively poor cutting speeds tabulated. The laser-induced reactions, evaporation and thermal effects touched on in chapters 7–9 are uniformly intriguing, and could interest a diverse selection of *Nature's* readership. These applications include: laser cleansing of surfaces, the deposition of thin films, chemical vapour deposition, surface studies and reactions, geological analyses, laser-triggered spark gaps, resistor-trimming, pyrolysis of coal and oil shale, gas-phase reactions and spectroscopy, crystal growth, metallurgical effects, and controlled stress generation in solids and liquids. This coverage is clearly too wide to permit detailed evaluation of specific topics; in particular, isotope separation and (thermal) surface heat treatment are both treated too briefly to be of interest to specialists in these fields. The book concludes with a chapter discussing propagation, atmospheric monitoring and the communications possibilities of the CO₂ laser. Pollution monitoring to parts in 10⁻⁹–10⁻⁸ using tunable diodes, spin-flip CO, CO and CO₂ lasers, and the back-scattering efficiency for typical particulate atmospheric constituents, are discussed in some detail. A systematic comparison of LIDAR performance using a range of laser sources is however, not attempted. Three appendices provide a useful compendium of thermal constants for a range of engineering materials.

The book is well produced, reasonably priced, and provides an interesting introduction and bibliography to the scientific and technical literature.

I. J. Spalding

I. J. Spalding works on laser applications at the UKAEA Culham Laboratory, Abingdon, UK.

Extraversion-introversion paradigm

The Measurement of Personality. By H. J. Eysenck. Pp. xviii+511. (MTP: Lancaster, 1976.) £12.50.

EYSENCK is remarkable in many ways, not least in his ability to generate books both as author and editor. This one, a collection of reprints, linked tightly and at length by editorial argument, deals almost entirely with the personality dimension of extraversion/introversion but has no overlap with the papers covered by his recent three-volume work on the same topic.

Nevertheless, the papers are carefully selected to exemplify and support the case for Eysenck's particular approach to the study of personality which he describes as a 'paradigm' in the Kuhnian sense of a professional or disciplinary matrix. This paradigm is closely modelled on physical science, depends on measurement and hypothetico-deductive procedures but goes beyond Popper to Lakatos' view of a research program which yields predictions of novel facts but tolerates anomalies among its findings; and in fact progresses from experimental analyses of these anomalies.

Eysenck's program is based on the claims that there is a set of invariant general traits underlying individual differences in behaviour, that these are explicable in terms derived from general experimental psychology, and that the broader and more important personality factors are likely to derive from more basic biological variables which, in turn, are largely determined by genetic factors. Apart from intellectual abilities he believes that there are only three higher order factors of this type. Extraversion/introversion (E) is one of these and the related behaviour is postulated to be a non-linear function of individual differences in characteristic levels of "cortical arousal". Sections of the book deal with models of personality, its physiological basis, differential reactions to pain and other sensations, and individual differences on vigilance tasks, in perceptual reactions, motor behaviour, learning, memory, fluency and creativity, and in social behaviour.

Many of Eysenck's opponents consider his objective, nomothetic approach inappropriate for the study of personality and engage in a holistic idiographic search for understanding, which he sees as fruitless. Others remain within his frame of reference but lay greater stress on the situational determinants of behaviour, which he

regards as largely irrelevant or as interfering factors to be controlled in his studies. He does recognise that they may have an important function in certain real life situations but even then stresses their interactions with an individual's personality. Other critics also quarrel with the heavy weighting Eysenck grants to heredity in his rather "either/or" approach to nature/nurture questions. A particular phenotype is the outcome of a developmental process in which experience may affect organismic maturation in a number of ways: by channelling it in certain directions but also by facilitating or inhibiting normal development. This is particularly true of early neural maturation, which plays an important role in Eysenck's theory.

The great strengths of Eysenck's personality program, however, are that it embraces more levels of functioning—biological, behavioural and social—in a more comprehensive way than any other and that it is closely related in an interactive developing fashion with an ongoing experimental research programme. In this book he certainly substantiates his claim to a "paradigm" and a theory in transition from "soft" to "hard". His critics should read this book with care: they will not find it easy to produce an equally powerful counter-argument.

H. Gwynne Jones

H. Gwynne Jones is Professor of Psychology at the University of Leeds, UK.

Solar flares

Solar Flares. By Z. Svestka. Pp. xiv+399. (D. Reidel: Dordrecht, The Netherlands and Boston, Massachusetts, 1976.) Cloth Dfl115; \$39.50; paper Dfl69; \$23.

THERE may be a temptation to regard Svestka's book as an update of Smith and Smith's work of the same title published fourteen years ago. To do so would, however, be to grossly underestimate the magnitude and success of Svestka's undertaking in this new book. For, firstly, the quantity of observational material to be reviewed has expanded since 1963 with a growth rate perhaps exceeded only by solar flares themselves. And, secondly, although the subject matter is intended primarily to be observational, an admirable awareness has been retained throughout of the need for objective interpretation in the areas of both classification and physical theory.

Approximately one-third of Svestka's efforts are devoted to the chromospheric flare as observed in optical wavelengths, and to flare-associated optical phenomena. Although this distribution may still reflect some of the historical overemphasis of hydrogen Balmer line observations, it seems well justified in view of Svestka's personal expertise in this area, and, also, of the observations being well tempered by theoretical discussion of such matters as inference of magnetic fields and models of chromospheric flare heating.

A comparable section is dedicated to "The High Temperature Flare" embracing not only the thermal XUV emissions but also the non-thermal hard X-ray and radio bursts. Much of this material appears here in mono-

graph form for the first time, having been born of the advances in space techniques over the past decade. The coverage given to Skylab and other recent results is remarkably good for a review of such a rapidly expanding field. Ample attention is again given to basic data interpretation and to detailed physical models where these exist—specifically for hard X-ray, microwave and Type III sources.

Following a chapter on particle emission, Svestka finally turns to the matter of flare models. In a sense, this is the weakest section since many different models are rather cursorily dealt with. This is not surprising since a second volume by the late L. D. de Feiter had been planned on purely theoretical aspects. Given, however, the current motley state of flare theory, and the complexity of the plasma physics involved, it is perhaps better simply to relate the broad features of models to observational manifestations rather than to attempt a survey of this difficult and incomplete area of theory.

Finally, it might have been appropriate to set the flare problem in a wider plasma astrophysical context such as theory of flare stars and energetic galactic phenomena. To do so would, however, have been to enter speculative realms not in keeping with the systematic objectivity which characterises this book and Svestka's work in general. This quality alone should suffice to remind astrophysicists of the intrinsic interest of the flare phenomenon, and certainly makes the book a must for all solar physicists.

J. C. Brown

J. C. Brown is Lecturer in the Department of Astronomy at the University of Glasgow, UK.

Biological effects of environmental agents

Biochemical Toxicology of Environmental Agents. By A. de Bruin. Pp.x+1544. (Elsevier/North Holland: Amsterdam and New York, 1976.) Dfl.320; \$130.75.

INTEREST in the biological effects of environmental agents has increased enormously in the past three decades, stimulated by the growing awareness of the potential threat to human health of the increasing number of chemicals in the environment. There has been a corresponding increase in the number of toxicological investigations (in man and experimental animals) and these have come to rely more and more on biochemical techniques both in the assessment of toxic effects and in the study of the mechanisms of toxicity.

Dr de Bruin's book provides in 42 chapters an up-to-date review of the many aspects of biochemical toxicology. The first chapters are fairly general in scope, dealing with the metabolism of foreign chemicals, with factors which modify metabolism and toxicity, and also with tests of exposure to toxic compounds. There follows a systematic treatment of the various aspects of toxicity which are considered in different chapters,

according to the biochemical or biological system which is affected. Thus, disorders of carbohydrate and lipid metabolism are first considered, then proteins, nucleic acids and so on, and, finally, immunological mechanisms and blood coagulation disorders. Each chapter has a short introduction outlining the normal biochemical principles relevant to the system, followed by the biochemical toxicology of chemical agents and the effect of ionising radiations.

Dr de Bruin's book will be a useful reference work. The amount of information presented is vast (about 13,000 references are quoted), and the book is unique in providing so much information on so many different aspects of biochemical toxicology in one single volume. That a single author should have been capable of covering so much ground on his own is in itself, a very considerable achievement.

Perhaps inevitably for a book of this size, treatment of the biochemical mechanisms of toxicity is sometimes superficial; the author presents all the data available in an exhaustive fashion with little critical appraisal, so that the reader is left without guidance as to which of the various findings reported are of particular significance.

F. De Matteis

F. De Matteis is a member of the scientific staff of the MRC Toxicology Unit, Carshalton, Surrey, UK.

Secrets of the Universe still locked up

The Universe. By J. Kleczek. Pp. vi+259. (Reidel: Dordrecht and Boston, Massachusetts, 1976.) Cloth Dfl.70; paper Dfl.43.

THE idea of a book about the Universe linking the large and the small (galaxies and elementary particles), and starting from particle physics and the four basic forces to build outward, is a good one. Unfortunately, this is not a good book. The principle reason for this is the severe tailoring required to fit a quart story into a one-pint volume; as the preface says, this is only achieved by giving no references, short-cutting arguments, expressing quantities often as orders of magnitude and keeping formulae to a minimum. As well as all that, the author's English is not always very readable, and the text could have been improved by some sympathetic editing.

Of all these shortcomings, the worst is that where several theories or hypotheses exist to explain some pheno-

menon—as is so often the case in studies of the Universe—the author chooses only one for discussion, and presents it uncritically. On the solar neutrino problem, the author's treatment is even more deficient; we are simply told that "Many suggestions have been made to explain the discrepancy. There is no lack of ideas", without being told what any of the ideas are. The confused reader will gain little comfort from the index, in which some topics are broken down in nit-picking detail, whereas others receive no mention; the entry under "B", for example, consists solely of "Big-Bang", with no mention even of black holes, although they are discussed in the text.

If the same material, arranged under the same chapter and section headings, had been expanded to twice the size of the present book and edited into smoother English, the result would have been a valuable addition to the astrophysical literature. As it is, the present volume adds nothing of any value and cannot be recommended.

John Gribbin

John Gribbin is a member of the Science Policy Research Unit, University of Sussex, UK.

Ecophysiology

Introduction to Physiological Plant Ecology. By P. Bannister. Pp. 273. (Blackwell Scientific: Oxford and London, 1976.) £4.75.

PLANT physiological studies have contributed, and continue to contribute a great deal to plant ecology, and many universities and colleges now teach undergraduate courses in ecophysiology. Many such courses have arisen from a desire to present plant physiology in its true evolutionary and environmental context. It is natural, therefore, that publishers have been anxious to provide suitable texts for such courses and the book reviewed here follows others by Etherington (*Environment and Plant Ecology*, Wiley, 1974) and Larcher (*Physiological Plant Ecology*, Springer, 1975).

Bannister covers the expected ground—energy balance, responses to light and temperature, soils, mineral and water relations and competitive interactions. The approach is concise and basic. He starts from first principles and relies mainly on verbal rather than numerical explanation of the principles discussed. This makes the text readable; also, it is rich in pertinent illustrations, largely drawn from British examples.

The attempt to portray such a wide and complex subject at an elementary level has necessitated a considerable degree of selection and pruning, and some topics are dealt with very briefly and may, consequently, be oversimplified. As a general criticism, I feel that too little information has been provided on relevant metabolic processes, which leaves one rather dissatisfied with some explanations. For example, an interesting collation of data concerning the tolerance of extreme temperatures by plant species stops short of explaining their metabolic consequences or the biochemical adaptations involved. At times the avoidance of biochemistry even obscures the text, as in the case of the C-4 photosynthetic system and the explanation of photorespiration. The same, however, applies to Etherington's book on this point.

Bannister's book stands out from the crowd by virtue of its rich use of examples of field problems which have been illuminated by physiological work. One can point students to it with the confidence that it will simulate their interest.

P. D. Moore

P. D. Moore is Lecturer in the Department of Plant Sciences at King's College, University of London, UK.

newly on the market

These descriptions are prepared by the staff of *Nature* on the basis of material provided by manufacturers. There is a Reader Enquiry Form facing page 28.

25 MHz oscilloscope. Pye Unicam. A 25 MHz oscilloscope with full digital measuring facilities found normally only on higher frequency models, the Philips PM 3214 has all the features of a general purposes oscilloscope with high 2 mV sensitivity over the full bandwidth. Digital measurements are facilitated by such features as a delayed timebase with fully calibrated delay and an alternate timebase facility which allows display of both main and delayed timebase signals at the same time for direct and easy reference. The wide range of power supply possibilities allows the oscilloscope to operate in the laboratory or the field. Mains supplies of 110, 127, 220 and 240 V a.c. $\pm 10\%$ at 46–440 Hz, 21–27 V d.c. supplies or an internal 24 V battery option are the alternatives. Power consumption of the oscilloscope is 30 W.

Circle No. 42 on Reader Enquiry Form

Fluorescence detector. Perkin-Elmer Ltd. A new fluorescence detector for liquid chromatography, LC-1000, has been designed with emphasis on high sensitivity, selectivity and ease of operation. The LC-1000 uses a xenon source providing a continuum over the UV/visible region. The square section quartz flow cell of 20 μl volume is designed to provide maximum sensitivity, minimum scatter and no detectable loss of peak resolution while still being simple to dismantle for cleaning. Model LC-1000 uses a continuous interference filter as an emission monochromator operating over the region 390–750 nm, and the ability to scan an emission spectrum of a peak in a chromatogram can provide peak identification—a unique advantage. For wavelengths below 390 nm the fluorescence can be monitored by any fixed interference filter.

Circle No. 43 on Reader Enquiry Form

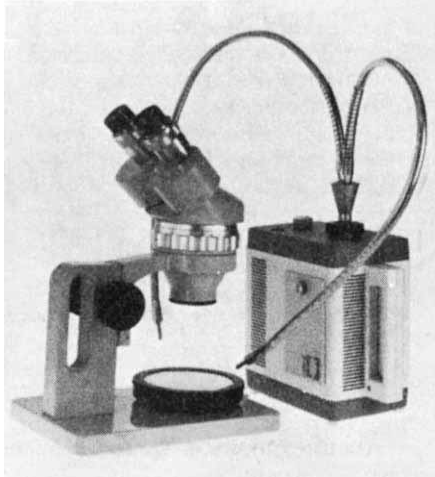
Photomultiplier power supply. EMI. The Type PM28A has the following characteristics from the electrical specification: output voltages from 100 to 2,800 V, ripple better than 2 mV peak-to-peak, temperature coefficient less than 50 p.p.m. per $^{\circ}\text{C}$ and load regulation 10 p.p.m. from a no load to full load (5 mA) change. An additional

feature is that the unit can be reset to within 0.2 V throughout the voltage range so that photomultiplier tube gains can be reproduced accurately. The unit is capable of positive or negative output, with options for remote programming and kits are available to mount units either singly or two abreast into a 19-inch rack.

Circle No. 44 on Reader Enquiry Form

Multiple sample harvesters. Ilacon Ltd. Designed for use in lymphocyte transformation assays, and a wide range of other isotope assays carried out in microplates, these harvesters introduce two unique design features which ensure trouble free and efficient operation. The basic model uses a patent disk cutter system to cut and hold the glass fibre filter paper, and a wash trough which allows complete versatility in washing without the need for switches or valves. Alternative models allow either supernatant collection or a disk clamping system for harvesting on to intact sheets of filter paper.

Circle No. 45 on Reader Enquiry Form



Will Z-33 zoom stereomicroscope.

Will Z-33 zoom stereomicroscope. Will-Strübin. The Will-Strübin stereo zoom microscope has a magnification range from $\times 2.7$ to $\times 180$. The eyepieces give extra wide field vision, and the eyepiece sleeves are adjustable and inclined 45° which guarantees comfortable vision in the normal sitting position. Various accessories are available including transmitted light universal stands, camera attachments, eyepieces $\times 5$, $\times 10$, $\times 15$, and $\times 20$, also auxiliary lenses and fibre optics.

Circle No. 46 on Reader Enquiry Form

Multipoint thermal printing recorder. International Instruments Ltd. The Speed Servo II combines a linear servo motor with a simple solid state semiconductor printing device that ensures positive multipoint channel identification. The printhead thermally brands the three basic types of readout: the channel number and a dot, all dots, or mixed dots and numbers as programmed. Models with up to 24 independent channels are available and the print rate can be as fast as one second per point full scale. The channel identification numbering system automatically prevents confusion when input values cause the traces to overlap, especially when all channels are in use, saving time in chart reading and analysis.

Circle No. 47 on Reader Enquiry Form

Liquid chromatographs. Perkin-Elmer. Series 2/1 is a single module providing both solvent delivery and sample injection and features high pressure capability, rapid solvent changeover, unlimited solvent supply, precise and steady flow, and a wide flow range adjustable from 0.1 ml min^{-1} to 29 ml min^{-1} for analytical and preparative separations. Series 2/2 combines the operating features of two Series 2/1 pumps and a sample injection system. The instrument provides linear solvent and flow programming capability from 0.1 ml min^{-1} to 30 ml min^{-1} . Series 2/2 also offers direct mixing of selected solvent compositions, preparative flow rates, and allows independent solvent changeover.

Circle No. 48 on Reader Enquiry Form

Bench-top chemostat. New Brunswick Scientific Co., Inc. This accurately defines growth parameters such as agitation, aeration, temperature, nutrient addition and harvesting of cells grown in pure culture. The BioFlo can also be equipped with miniature probes and controllers for dissolved oxygen and pH regulation. With a working volume of only 350 ml, it is possible to sustain cell growth for a minimum of 38 h without replenishing the medium. The BioFlo can be operated at temperatures from below ambient to 60°C . A magnetically coupled agitator produces a homogeneous dispersion of culture medium under aseptic conditions at speeds from 200 to 1,000 r.p.m.

Circle No. 49 on Reader Enquiry Form

announcements

Meetings

18–19 May, **Evolutionary Systematics of Bivalve Molluscs**, London (P. B. Carter, The Royal Society, 6 Carlton House Terrace, London SW1).

20 May, **Digital Satellite Communications**, London (The Institution of Electrical Engineers, Savoy Place, London WC2).

25 May, **World Energy Prospects to the Year 2000**, London (P. B. Carter, The Royal Society, 6 Carlton House Terrace, London SW1).

30 May–3 June, **American Geophysical Union Annual Meeting**, Washington, D.C. (AGU, Sheraton Park, Woodley Road, N.W., Washington, D.C. 20008).

1–2 June, **Essential Fatty Acids and Disease**, London (Royal Society of Medicine, 1 Wimpole Street, London W1).

4–6 July, **Rare Earths and Actinides**, Durham (The Institute of Physics, 47 Belgrave Square, London SW1).

10–22 July, **Sensory Ecology—Nato Advanced Study Institute**, Quebec (M. A. Ali, Biologie, Université de Montréal, Case Postale 6128, Montréal).

11–13 July, **Physical Chemistry and Hydrodynamics**, Oxford (D. Brian Spalding, Mechanical Engineering, Imperial College, Exhibition Road, London SW7).

13–15 July, **Processing, Structure, Properties and Performance of Polymers**, Nottingham (The Institute of Physics, 47 Belgrave Square, London SW1).

13–15 July, **3rd International Workshop on Phosphate and other Minerals**, Madrid (A. Rapado, Unidad, Metabolica, Fundacion Jimenez Diaz, Av. Reyes Catolicos, 2, Madrid-3, Spain).

18–29 July, **Energy for Energy Decision Makers**, Cambridge, MA (Room E19-356, MIT, Cambridge, MA 02139).

18–23 July, **XXVII International Congress of Physiological Sciences**, Paris (J. Scherrer, U.E.R. Pitie-Salpetrière, Cedex 1300, F-75300, Paris).

24–30 July, **Optimal Development and Management of Groundwater**, Birmingham (J. W. Lloyd, International Association of Hydrogeologists, Geological Sciences, University of Birmingham, UK).

24–30 July, **40th Annual Meeting of the Meteoritical Society**, Cambridge (E. Scott, Mineralogy and Petrology, Downing Place, Cambridge, UK).

25–29 July, **6th AIRAPT International High Pressure Conference—Applications and Techniques**, Boulder (K. D. Timmerhaus, Engineering Research Centre AD-1-25, University of Colorado, Boulder, Colorado 80309).

25–28 July, **Synthesis in Organic Chemistry**, Oxford (J. F. Gibson, The Chemical Society, Burlington House, London W1).

26–29 July, **Electron Transport/Molecular Solids**, Leeds (G. J. Morgan, Physics, University of Leeds, UK).

2–5 August, **Cryogenics—A Century of Progress, A Challenge for the Future**, Boulder (K. D. Timmerhaus, University of Colorado, Boulder, Colorado 80302).

7–13 August, **8th International Conference on General Relativity and Gravitation**, Waterloo, Ontario (M. A. McKiernan, Mathematics, University of Waterloo, Ontario, Canada).

21–27 August, **5th International Conference on Birth Defects**, Montreal (International Birth Defects Congress Ltd, 1275 Mamaronek Ave, White Plains, New York 10605).

22–26 August, **International Symposium on Microbial Ecology**, Dunedin, New Zealand (The Royal Society of New Zealand, PO Box 12249, Wellington, N.Z.).

22–24 August, **Symposium on Scientific Instruments: Their Social and Economic Settings**, Greenwich (Navigation and Astronomy, National Maritime Museum, Greenwich, London SE10).

29 August–2 September, **48th ANZAAS Congress**, Melbourne (Secretariat, 48th ANZAAS Congress, PO Box 29, Parkville, Victoria, Australia 3052).

29 August–1 September, **6th International Symposium on Drugs Affecting Lipid Metabolism**, Philadelphia (W. L. Holmes, Lankenau Hospital, Philadelphia, Pennsylvania 19151).

30 August–1 September, **Dopamine**, Southampton (P. J. Roberts, Physiology and Biochemistry, University of Southampton, UK).

30 August–2 September, **Biological Activity and Chemical Structure**, Noordwijkerhout (Netherlands) (Secretary, c/o Merck Sharpe and Dohme B.V. Waarderweg 39, PO Box 581, Haarlem, Netherlands).

30 August–3 September, **4th European Crystallographic Meeting**, Oxford (C. K. Prout, Chemical Crystallography Laboratory, 9 Parks Road, Oxford).

31 August–2 September, **1st New Zealand Astronomy and Astrophysics Conference**, Christchurch (Secretary, Physics, University of Canterbury, Private Bag, Christchurch, N.Z.).

4–9 September, **13th International Symposium on Free Radicals**, Lyndhurst, Hampshire (A. Carrington, Chemistry, The University, Southampton, UK).

4–9 September, **4th International Congress on Photosynthesis**, Reading (J. Coombs, c/o Tate and Lyle Ltd, P. Box 68, Reading, UK).

5–8 September, **New Processes of Waste Water Treatment and Recovery**, London (D. H. Sharp, Society of Chemical Industry, 14 Belgrave Square, London SW1).

5–10 September, **4th International Round Table on Sintering**, Dubrovnik (ETAN, PO Box 356, 11001 Beograd, Yugoslavia).

Person to Person

Exchange 4 bedroom house just south of Cambridge, England for a house conveniently situated for Stanford University, California for a period of one year beginning August/September. Please contact Dr Peter Twentyman, MRC Clinical Oncology Unit, Hills Road, Cambridge.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

8–19 August, **Physics of Quantum Electronics**, Telluride, Colorado (S. F. Jacobs, Rt. 2, Box 732D, Tucson, Arizona 85715).

10–19 August, **XVth International Congress of the History of Science**, Edinburgh (E. G. Forbes, The Royal Society of Edinburgh, 22 George Street, Edinburgh, UK).

11–13 August, **Energy Conservation in Crop Production**, Palmerston North, New Zealand (Agronomy, Massey Department, Palmerston North, N.Z.).

15–18 August, **5th International Conference on Chemical Thermodynamics**, Ronneby, Sweden (Chemical Centre, Lund University, P.O.B. 740, S-220 07 Lund, Sweden).